UNIVERSITY OF LIVERPOOL

THE EFFECT OF

APPETITE ON PAIN

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

Frequent abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
BMI	Body mass index
BOLD	Blood oxygenation level dependent
CLARA	Classical LORETA analysis recursively applied
CSF	Cerebrospinal fluid
DMN	Default mode network
EEG	Electroencephalography
EPI	Echo planar imaging
FDR	False discovery rate
FEP	Fluorinated ethylene propylene
FMRI	Functional magnetic resonance imaging
FWE	Family-wise error
HRF	Hemodynamic response function
IFG	Inferior frontal gyrus
IPC	Inferior parietal cortex
LEP	Laser evoked potential
LORETA	Low resolution brain electromagnetic tomography
MFG	Middle frontal gyrus
MNI	Montreal Neuroimaging Institute
MRI	Magnetic resonance imaging
Nd-YAP	Neodymium-doped yttrium aluminium perovskite
OFC	Orbitofrontal cortex
PAG	Periaqueductal grey
PCC	Posterior cingulate cortex
PFC	Prefrontal cortex
PHG	Parahippocampal gyrus
POMS	Profile of mood states

RF	Radiofrequency
ROI	Region of interest
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SFG	Superior frontal gyrus
SPC	Superior parietal cortex
TE	Time to echo
TFEQR18	Three Factor Eating Questionnaire - Revised
TR	Time to repeat
VAS	Visual analogue scales
VB	Ventrobasal

Contents

List of Figures

List of Tables

This thesis is submitted in fulfilment of the conditions for a PhD by published papers. In accordance with the University of Liverpool guidelines and regulations the experimental chapters (Chapters 3 to 7) of this thesis will take the form of journal article manuscripts, which have either been published during the preparation of this thesis, are under review in a peer-reviewed journal, or are being read by co-authors before submission to a peer-reviewed journal. Specific details with regards to journal submission and contribution of authors are given at the beginning of each chapter, as required.

The effect of appetite on pain

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Abstract

Hunger and pain are powerful homeostatic drives, which compete for a behavioural response when experienced simultaneously. This thesis set out to explore neural mechanisms underpinning this competition, and how appetitive visual and olfactory stimuli may modulate the effect of homeostatic energy manipulations on pain. Using well-established techniques including EEG source analysis and resting state fMRI, we consistently employed a within-subjects fasting vs. satiation paradigm to investigate the effects of appetite on subjective pain perception and neural pain processing. Pain stimuli which selectively activated nociceptive Aδ fibres were presented concurrently with appetitive stimuli, and the neural nociceptive responses were mapped with high-density (128-channel) EEG recordings and fMRI functional connectivity. Based on the results of previous research, we hypothesised that fasting would suppress subjective and neural pain processing, and that visual and olfactory appetitive stimuli may augment this effect.

We first found that a relatively short overnight fast was sufficient to induce significant changes in resting state functional connectivity in areas that underlie both hunger / satiety and pain: insula cortex, hypothalamus, and regions of prefrontal cortex. Source analysis of EEG data revealed a small group of brain regions whose pain-related activation was suppressed by hunger and / or appetitive stimuli: anterior cingulate cortex, operculo-insular cortex, parahippocampal cortex, and cerebellum. Functional connectivity analysis of fMRI data further uncovered a widely-distributed network of brain areas whose pain-induced connectivity was enhanced by fasting or satiety. Of particular interest was a small network of areas involved in stimulus saliency processing (anterior insula, anterior cingulate cortex, and prefrontal cortex), which was stronger during fasting; presumably advantageous when searching for food. Lastly, in an experiment using a bread odour, we found that the suppressive effect of appetitive stimuli on nociception is not just confined to the visual modality. Brief, strong pain can also be suppressed by an appetitive odour during fasting. We conclude that fasting reliably interferes with pain processing, and that ambient appetitive stimuli might be of use in situations where short-lasting pain is likely to occur.

Declaration

No portion of this work has been submitted in support of any other application for degree or qualification at this or any other University or institute of learning.

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In memory of Peter Wright, 1948 – 2006.

Chapter 1

General introduction

"We are all ruled in what we do by impulses ... Hunger, love, pain, fear are some of those inner forces which rule the individual's instinct for self-preservation." – Einstein (1950).

Pain is a significant societal problem, with approximately 31 million days of work lost to musculoskeletal problems during 2013 in the UK (ONS 2014). Consequences of chronic pain for patients include job loss and depression, seriously impacting on quality of life. While pharmacological treatment remains the standard, many patients also undergo therapies such as massage and acupuncture (Breivik et al. 2006). Since there is a sizeable cohort of patients willing to consider treatments in addition to pharmacotherapy, it would be useful to explore a benign and potentially useful adjunct to current treatment plans: homeostatic energy balance manipulation in combination with salient food cues. Animal and human experiments on hunger's potentiating or moderating influence over pain have been carried out (albeit somewhat disjointedly) over the past few decades, with some encouraging findings. A better understanding of cortical interactions between hunger and pain could enable novel treatment approaches; this is the overarching motivation behind the thesis.

Pain is not merely a sensation provoked by a noxious stimulus. It is a vital homeostatic mechanism (Craig 2013; 2003b); it is therefore logical to hypothesise that other salient stimuli related to homeostasis would compete with pain for limited attentional resources. In support of this theory research demonstrates that in humans, experiencing breathlessness inhibits pain perception (Morelot-Panzini et al. 2007; Nishino et al. 2008; Yashiro et al. 2011), while thirst (Farrell et al. 2006) and sleep deprivation (Azevedo et al. 2011; Kundermann et al. 2004; Onen et al. 2001; Schuh-Hofer et al. 2013; Tiede et al. 2010) enhance pain perception. The interaction between pain and hunger is explored during the following chapters.

The following sections in this general introduction describe the physiological pathways of pain, hunger, taste, and olfaction. Next, overlapping brain areas which represent potential sites for interactions between competing drives are highlighted, and results of literature searches for previous studies examining the interaction between appetite / appetitive stimuli and pain, and vice versa are reported.

1.1 Neural mechanisms of pain

Fast pain is transmitted by Aδ fibres, and follows a complex pathway. Pain signals from the skin are transmitted to the spinal cord by small Aδ fibres, which terminate mainly in lamina I of the dorsal horns. The signal is transmitted on up the spinal cord, via fibres in the neospinothalamic tract, to areas of the brainstem (ventrolateral medulla, parabrachial nucleus, and periaqueductal grey matter: PAG). Some of these fibres terminate in the brainstem, but the majority proceed to the thalamus (Craig 1995). The signal is passed on from the thalamus to the somatosensory cortex, and also from the thalamus to hypothalamus and amygdala, both of which project back to the thalamus. Ascending signals from lamina I are also passed directly from the thalamus to posterior insula, and then re-represented in middle and anterior insula. Lamina I signals activate anterior cingulate cortex (ACC) and area 3a of primary somatosensory cortex (S1; Craig 2003b).

The pain pathway also permits descending modulation of pain; insula modulates the brainstem, and the brainstem and hypothalamus both descendingly modulate laminar I activity. Descending modulation is constantly active under normal circumstances (Diesel et al. 1990; Wilson et al. 2002).

The PAG in several species contains opiate receptors, and injecting opiates into the PAG produces behavioural analgesia (Fields and Basbaum 1978) . Stimulation of the PAG in the brainstem can produce powerful inhibition of pain signalling. In rats, electrical stimulation obviated the need for a general anaesthetic when the animals underwent abdominal surgery (Reynolds 1969) . In rat models of neuropathic pain, deep brain stimulation of the PAG produced effective analgesia (Lee et al. 2012), and reduced mechanical and cold allodynia (Lee et al. 2000). The nociceptive role of the PAG has not been as thoroughly explored in humans as in rats, due both to the

invasiveness of stimulation procedures, and to the difficulties encountered in imaging this area due to signal drop-out. However, some studies of electrical stimulation of PAG to relieve chronic pain unresponsive to other treatments were carried out in the 1970s and 80s. Analgesic results were highly variable across studies, due to a lack both of standardised pain relief criteria and of standardised electrode implantation sites (Carrive and Morgan 2003) . Additionally, there were many accompanying aversive effects that were so unpleasant that many patients could not tolerate the stimulation, and discontinued their treatment (Boivie and Meyerson 1982) . The aversive side effects were often reported as sensations of fear and panic, which, considered along with animal evidence showing that PAG is activated when the 'fight or flight' response is triggered (Jansen et al. 1998), points to a role for the PAG in co-ordinating behavioural responses to threat or injury and the necessary accompanying analgesia.

The PAG projects to the rostroventral medulla; anaesthetic or damage to which renders the antinociceptive signalling from PAG ineffective (Behbehani and Fields 1979; Gebhart et al. 1983) . Along with the nucleus raphe magnus, these structures form a descending pathway from the brain that inhibits pain perception (Vanegas and Schaible 2004). Conversely, while the rostroventral medulla does not appear to be necessary for initialising neuropathic pain, it maintains neuropathic pain by descending facilitation (Burgess et al. 2002).

The thalamus is involved in both the sensory and the affective aspects of pain. In monkeys, neurons responding specifically to noxious stimuli project from lamina I of the dorsal horn to ventrobasal (VB) thalamus (Ralston and Ralston 1992); approximately 50% of VB neurons are responsive to noxious stimuli (Chung et al. 1986). In humans, the thalamic VB region homologous to monkey VB has also been found to contain neurons that respond to noxious stimuli (Lenz et al. 1994). In patients with chronic pain due to peripheral or central neural damage, microstimulation of VB produces sensations of burning pain (Lenz et al. 1993). Traditionally it was assumed that medial thalamus was involved only in emotional response to pain while lateral thalamus dealt with sensory aspects of pain, but it has been demonstrated that some neurons in medial thalamus code the intensity and duration of painful stimuli (Bushnell and Duncan 1989).

Nociceptive projections from the ventral posterolateral nucleus of the thalamus innervate S1 (Gingold et al. 1991; Kenshalo et al. 1980). Animal S1 contains neurons that respond to nociceptive input, though they are outnumbered by neurons that respond to innocuous tactile stimuli. The S1 nociceptive neurons are somatotopically organised (Kenshalo et al. 2000; Omori et al. 2013), and are strongly involved in sensory-discriminative aspects of pain, coding the actual intensity (Chen et al. 2009; Ohara et al. 2004a), perceived intensity (Kenshalo et al. 1980), duration (Kenshalo and Isensee 1983), and location (Bushnell et al. 1999) of pain. Direct subdural recordings from S1 neurons in monkeys have identified responses to nociceptive heat stimuli (Apkarian et al. 2005; Hofbauer et al. 2001; Lenz et al. 2010) and tooth pulp stimuli (Price 2000). Human subdural recording studies are relatively rare, but such studies have found that neurons in human S1 respond to nociceptive laser stimuli (Baumgartner et al. 2011; Kanda et al. 2000; Ohara et al. 2004b). Lesions to S1 appear to cause gross disturbances in the ability to pinpoint the location of pain (Ploner et al. 1999a).

Secondary somatosensory cortex (S2) receives direct nociceptive projections from the ventral posterior inferior nucleus of the thalamus (Friedman et al. 1986; Stevens et al. 1993). While subdural recordings from S2 have identified a small number of neurons that respond to pain, their receptive fields are large and bilateral and they generally do not code stimulus intensity well (Dong et al. 1989; 1994; Robinson and Burton 1980). It seems odd, then, that a number of imaging studies have concluded that S2 plays an important sensory-discriminative role in pain (Maihofner et al. 2006; Maihofner and Kaltenhauser 2009; Worthen et al. 2011), even coding pain intensity (Coghill et al. 1999; Tseng et al. 2010; though see Tran et al. 2010 for contradictory findings). A plausible explanation for this discrepancy is that the nociceptive area for S2 is located just outside the area usually designated as S2, and so subdural recording experiments may have actually missed the nociceptive neurons (Schnitzler and Ploner 2000). In response to noxious stimuli S2 is activated in parallel to S1 (Liang et al. 2011; Ploner et al. 1999b), and is thought to be important in pain-associated learning and memory processes (Schnitzler and Ploner 2000), and pain anticipation (Seifert et al. 2012).

Anterior insula receives projections from the posterior area of the thalamic ventral medial nucleus (Craig 1995). This nucleus is itself projected to by lamina I of the spinal cord dorsal horn, and the majority of neurons contained therein respond to

nociceptive and thermoreceptive stimuli (Craig et al. 1994). Subdural recordings have found nociceptive neurons in insula cortex (Zhang et al. 1999), and specifically in both anterior insula (Dostrovsky and Craig 1996) and posterior insula (Robinson and Burton 1980). Insula also receives afferents from S2 and projects to amygdala and hypothalamus (Friedman et al. 1986; Mesulam and Mufson 1985; Shi and Cassell 1998a; 1998b), and is therefore ideally placed to integrate nociceptive signals before passing them on to the limbic system. It is also postulated to play a role in pain-related memory and learning (Albanese et al. 2007; Schnitzler and Ploner 2000). There are many imaging studies that have employed painful stimuli, and the vast majority report that anterior insula is activated in response to pain. Anterior insula is involved in coding the threat of pain (Franciotti et al. 2009) and anticipation of pain (Chua et al. 1999; Ploghaus et al. 1999; Porro et al. 2002), and its activation before a threshold stimulus is delivered can predict whether or not the stimulus will be perceived as painful (Ploner et al. 2010). It is also activated in response to other people experiencing pain (Jackson et al. 2005; Singer et al. 2004). There is a critical role for posterior insula cortex in pain perception (Favilla et al. 2014; Garcia-Larrea 2012; Mazzola et al. 2012; Segerdahl et al. 2015; Wiech et al. 2014).

The ACC receives nociceptive signals from lamina I of the spinal cord, via medial thalamus (Craig 2003c). It contains neurons that respond only to nociceptive stimuli, which, while demonstrating some intensity coding for noxious heat stimuli (Bushnell and Duncan 1989; Sikes and Vogt 1992) and painful mechanical stimuli, do not appear to be much involved in the sensory-discriminative aspects of pain due to their large receptive fields that encompass much of the body surface (Treede et al. 1999). The ACC is part of the limbic system, sharing many reciprocal connections with the amygdala (Mega et al. 1997), and as such may be assumed to be involved in emotional aspects of pain. In support of this observation, ACC is involved in anticipation of pain (Koyama et al. 1998), attention to and escape from pain (Iwata et al. 2005), pain avoidance (Koyama et al. 2001; Shyu et al. 2008), pain unpleasantness (Rainville et al. 1997), and fear conditioning (Feng et al. 2013; Tang et al. 2005). There is also a report of single neuron testing in humans (Hutchison et al. 1999); in agreement with the animal literature, the neurons identified have large and sometimes bilateral fields. Some ACC neurons responded specifically to nociceptive mechanical and hot / cold stimuli, and also to anticipation of pain and watching painful stimuli being applied

to another person. Interestingly, electrical stimulation applied to ACC failed to produce painful sensations. As the authors note, explanations for this could include the absence of co-activation of S1 or insula, that electrical stimulation may be too dissimilar from the normal cell firing patterns, and that the ACC neuronal activation previously elicited may actually represent a descending modulation of pain, not pain perception *per se*. Damage to the cingulate cortex or its white matter connections does not impair the ability to detect painful stimuli; rather, it removes the motivational and affective components from pain (Corkin and Hebben 1981; Foltz and White 1962; Hurt and Ballantine 1974; Vaccarino and Melzack 1989). An fMRI paper on painful stimulus-response functions (Bornhovd et al. 2002) found that activations in other areas reflecting stimulus, pain, attention, and working memory processes were also represented in subregions of ACC, indicating that the ACC functions as an integration centre for different aspects of pain perception.

In the overwhelming majority of papers on nociception, the amygdala is found to play a role in pain. The S1 does not have many projections directly to the amygdala; most somatosensory information reaches the amygdala indirectly via insula cortex and thalamus (Sah et al. 2003). It is reciprocally connected with the hypothalamus (Renaud and Hopkins 1977) and the ACC (Amaral and Price 1984; Mega et al. 1997). Subdural recordings have found neurons that respond to nociceptive stimuli in the amygdala (Bernard and Besson 1990; Bernard et al. 1992; Neugebauer and Li 2002), and the structure is thought to be important for producing antinociception (Carrasquillo and Gereau 2007; Huang et al. 1993; Rouwette et al. 2012). As part of the limbic system it is robustly implicated in affective responses to and affective modulation of pain (Ji and Neugebauer 2008), activating for example in response to uncertainty about pain (Bornhovd et al. 2002), and pain-related anxiety (Ji et al. 2007).

Hypothalamic activation has been demonstrated in response to experimentally-induced acute heat pain (Dube et al. 2009) and cold pressor pain (Petrovic et al. 2004). Deep brain hypothalamic stimulation had been utilised to treat drug-resistant cluster headaches in 38 patients up to July 2008, which resulted in 61% of these patients reporting that they were completely or almost pain free at a minimum of 7 months follow up (Leone et al. 2008). It is likely that the hypothalamus is a top-down modulator of pain, involved in terminating rather than precipitating pain (at least in cluster headaches), and that stimulating it restores its ability to control nociception (May et al.

2006), seemingly over a number of years (Leone et al. 2013; Piacentino et al. 2014). Whether the same holds true for other chronic pain conditions or acute experimentally-induced pain remains to be elucidated; success has been reported when using hypothalamic deep brain stimulation to treat other types of headaches (Bartsch et al. 2011; Leone et al. 2005; Lyons et al. 2009; Walcott et al. 2009), but was unsuccessful in treating a small number of patients with atypical facial pain (Broggi et al. 2007).

1.2 Physiology of hunger and taste

The hunger / appetite pathway begins with vagal afferents from the stomach projecting to the nucleus tractus solitarius in the brainstem. The signal is passed on to several hypothalamic nuclei: the paraventricular nucleus, ventromedial nucleus, dorsomedial nucleus, lateral hypothalamic area, and arcuate nucleus. The arcuate nucleus projects to the amygdala and nucleus accumbens, which in turn projects to the prefrontal cortex. The lateral hypothalamic area sends afferents to the cortex, which also receives input directly from the brainstem.

The brainstem is principally involved in the mechanics of feeding behaviour; sectioning an animal above the brainstem and below the hypothalamus does not prevent the animal from eating, nor does it eliminate the animal's ability to decide whether to swallow or reject possibly noxious food (Grill and Norgren 1978). Lateral hypothalamic neurons are modulated by hunger, and in addition, the paraventricular and ventromedial nuclei of the hypothalamus give rise to satiety. Damage to these nuclei causes extremely excessive eating; while damage to the dorsomedial nucleus causes aphagia (Guyton and Hall 2004). The amygdala is crucial for learned taste aversion, as well as being involved in learning to associate taste reinforcers with other arbitrary stimuli (Sanghera et al. 1979). Nucleus accumbens (part of the ventral striatum), is an area with a strong concentration of dopaminergic neurons. It appears to be important for learning to associate the rewarding value of food with motor activity necessary to obtain it (Williams et al. 1993), and for integrating cognitive, sensory, and emotional information about food with signals from the hypothalamus (Kelley 2004).

In addition to the areas cited above, neurons that respond to taste have been identified in the ACC (de Araujo et al. 2003a; Kringelbach et al. 2004), and neurons

responding to taste intensity have been found in the cerebellum, pons, and middle insula (Small et al. 2003). The ACC, orbitofrontal cortex (OFC), and middle insula also code for taste pleasantness (de Araujo et al. 2003b).

It is important to consider the role of taste in appetite. If it were merely homeostatic processes that controlled food ingestion, there would not be the prevalence of obesity due to people over-consuming high calorie foods. The hedonic value of food is a highly significant factor in eating behaviour. The taste pathway (described in Kringelbach et al. 2004) is comprised of many areas. Taste information is relayed from sensors on the tongue to the brainstem, and on to the medial nucleus of the thalamus (Pritchard et al. 1989). It is then passed on to the primary taste cortex in the frontal operculum, dorsal anterior insula (Pritchard et al. 1986; Scott et al. 1986; Sudakov et al. 1971), and area 3b of S1 (Norgren 1990). Primary taste cortex projects to secondary taste cortex (Baylis et al. 1995), and to lateral hypothalamus (Burton et al. 1976). Neurons responding to taste are also present in amygdala (Sanghera et al. 1979), and ventral striatum (including nucleus accumbens: Williams et al. 1993).

The gut-brain axis, the association between gastrointestinal tract and brain structures (hypothalamus and brain stem), is influenced by a variety of neurochemical entities which can be orexigenic (hunger-inducing), or anorectic (satiety-signalling). Most act either directly on the arcuate nucleus of the hypothalamus, or indirectly via vagal afferents, through the brainstem (nucleus of the solitary tract), and on to the hypothalamus (Wren and Bloom 2007). An exhaustive review of these factors is not the aim of this introduction, so only the most commonly cited will be discussed here.

The only circulating orexigenic factor is ghrelin. It is produced in the stomach, and acts on the hypothalamus both directly (Willesen et al. 1999), and indirectly via the vagus nerve (Asakawa et al. 2001; Date et al. 2002). Ghrelin appears to function in the short-term as a meal initiator, and its circulating levels are strongly correlated with subjective hunger ratings (Cummings et al. 2004). It also seems to be important for long-term weight maintenance; levels are increased when weight decreases (Adams et al. 2011; Weigle et al. 2003), and decreased when weight increases (Otto et al. 2001; Tschop et al. 2001).

Other known neurochemical appetitive factors are anorectic (satiety signalling), and are circulated after feeding. Cholecystokinin, peptide YY, glucagon-like peptide-1,

and oxyntomodulin are released from the intestine, and all reduce calorie intake in rodents and humans (Chaudhri et al. 2008). Additionally, administration of oxyntomodulin to obese human subjects increases their calorie expenditure by raising their physical activity levels towards normal (Wynne et al. 2006). Pancreatic polypeptide is released from the pancreas, and acts in the short-term to suppress appetite after feeding. Administering pancreatic polypeptide to human subjects can also reduce food intake for the subsequent 24 hours (Batterham et al. 2003).

In contrast to the acute-acting factors described above, the hormone leptin is produced in adipose tissue, and plays an important role in the long-term regulation of energy balance. Leptin concentrations are proportional to the amount of adipose tissue. It prompts the down-regulation of orexigenic compounds and the up-regulation of anorectic factors when fat stores are increased (Jequier 2002).

1.3 Neurochemical appetite modulators and pain

The administration of ghrelin has a strong pain relieving effect in animal models of neuropathic and acute pain (Guneli et al. 2010; Sibilia et al. 2006; Wei et al. 2013; Zeng et al. 2014; Zhou et al. 2014), as does glucagon-like peptide-1 (Gong et al. 2014), and neuropeptide Y (Hua et al. 1991; Li et al. 2005), an orexigenic compound expressed in the hypothalamus. Other research has found that leptin can enhance nociception (Kutlu et al. 2003; Lim et al. 2009; Tian et al. 2011), though one study has reported anti-nociceptive effects of leptin administration (Li et al. 2013). These substances are also found in brain areas known to be critically involved in nociception (Attele et al. 2002; Ferrini et al. 2009; Li et al. 2005; Vergnano et al. 2008; Zheng et al. 2014). It seems clear that these compounds play a prominent role in both maintaining the homeostatic energy balance, and analgesia / pro-nociception.

1.4 Physiology of olfaction

Olfactory signalling is projected from the olfactory bulb to primary olfactory cortex (O1). It diverges from O1 to OFC, insula, thalamus, and hippocampus (Fioretti et al. 2011). From OFC it passes to amygdala (Rolls 2004), hypothalamus (Mai and Paxinos

2011), ACC (Rolls 2012), and insula (Gottfried and Zald 2005). Amygdala sends olfactory projections to the hypothalamus, and thalamus projects back to OFC (Fioretti et al. 2011).

Food odours are responded to differently than non-food odours, and this modulation varies according to satiety status; after consuming a meal, participants reported a decrease in the pleasantness of food odours but no decrease in response to non-food odours (Albrecht et al. 2009; Duclaux et al. 1973). The pattern of responses that would suggest such alliesthesia has also been observed in rats (Pager et al. 1972). Olfactory bulb cells in rats respond differently to food odours according to the animal's satiety status, but satiety does not affect their response to non-food odours (Chaput and Holley 1976). Some neurons in monkey OFC have been found to decrease their firing rate in response to the smell of a food fed to satiety, while retaining or increasing their firing rate in response to the smell of other food and non-food odours (Critchley and Rolls 1996); decreased activation has also been found in human OFC in response to the smell of a food (banana) eaten to satiety, but there was no such decrease in response to another food odour (O'Doherty et al. 2000). Studies with human participants that utilise food odours are exceptionally rare, but show that food odours hold a special significance in comparison to other biologically irrelevant odours (Boesveldt et al. 2010; Kemmotsu and Murphy 2006; Small et al. 2007) and are responded to preferentially in limbic and reward-related brain areas (Bragulat et al. 2010). The administration of the orexigen ghrelin to fasted participants results in enhanced sniffing of both food and non-food odours (Tong et al. 2011), which presumably is a homeostatically advantageous behaviour. Recently it has been demonstrated that humans are even able to detect fat concentration by olfaction alone, which likely would have been beneficial for survival when food resources were scarce (Boesveldt and Lundstrom 2014).

1.5 Olfaction and pain

Unsurprisingly, studies on pain and olfaction are rare. Those that exist mainly used rats as subjects and lemon oil as the olfactory stimuli, and found that lemon oil aroma significantly reduces pain-related behaviour and modulates pain-induced neurochemical release in a variety of brain structures (Aloisi et al. 2002; Ceccarelli et al. 2002; Ikeda et

al. 2014). Rather than being immediately engaging to homeostatic brain regions (obviously lemons are edible, but hunger would not drive most animals to eat one), lemon oil aroma appears to affect limbic structures; destroying the ACC abolishes the aroma-induced suppression of pain-related behaviour and modulation of pain-induced neurochemical release (Ikeda et al. 2014).

Research with human participants has produced mixed results, with some studies finding that pleasant odours reduced pain perception (Aou et al. 2005; Bartolo et al. 2013; Demers et al. 2004), even when participants were not consciously aware of an odour manipulation taking place (Leduc et al. 2007), and one study finding a pro-nociceptive effect of unpleasant odours (Bartolo et al. 2013). Other findings contradicted these results (Marchand and Arsenault 2002; Martin 2006). The only available pain study with adult human participants and food odours found that a sweet caramel odour significantly increased pain tolerance, while a pleasant non-food odour (aftershave) did not (Prescott and Wilkie 2007). The authors interpret these results as being due to a conditioned association between sweet odours and sweet tastes. In spite of the lack of previous research in this area, it is logical to assume that olfactory food stimuli constitute powerful behavioural motivators, and may therefore have robust effects on nociception.

1.6 Pathway interactions

Figure 1 shows abridged connectivities of the pain, hunger, taste, and olfaction pathways. Thalamus and hypothalamus are common to all four pathways; the brainstem projects hunger and taste signals and has pain pathway afferent and efferent connections; insula is a target of olfaction, taste, and pain pathways, and also projects pain signals to several other areas; the hunger and olfaction pathways pass through amygdala, and amygdala carries many afferent and efferent projections of the pain pathway. The ACC is a target of the pain and olfactory pathways and is often cited as an area activated by hunger (Fuhrer et al. 2008; Hinton et al. 2004; Tataranni et al. 1999), possibly due to negative affect.

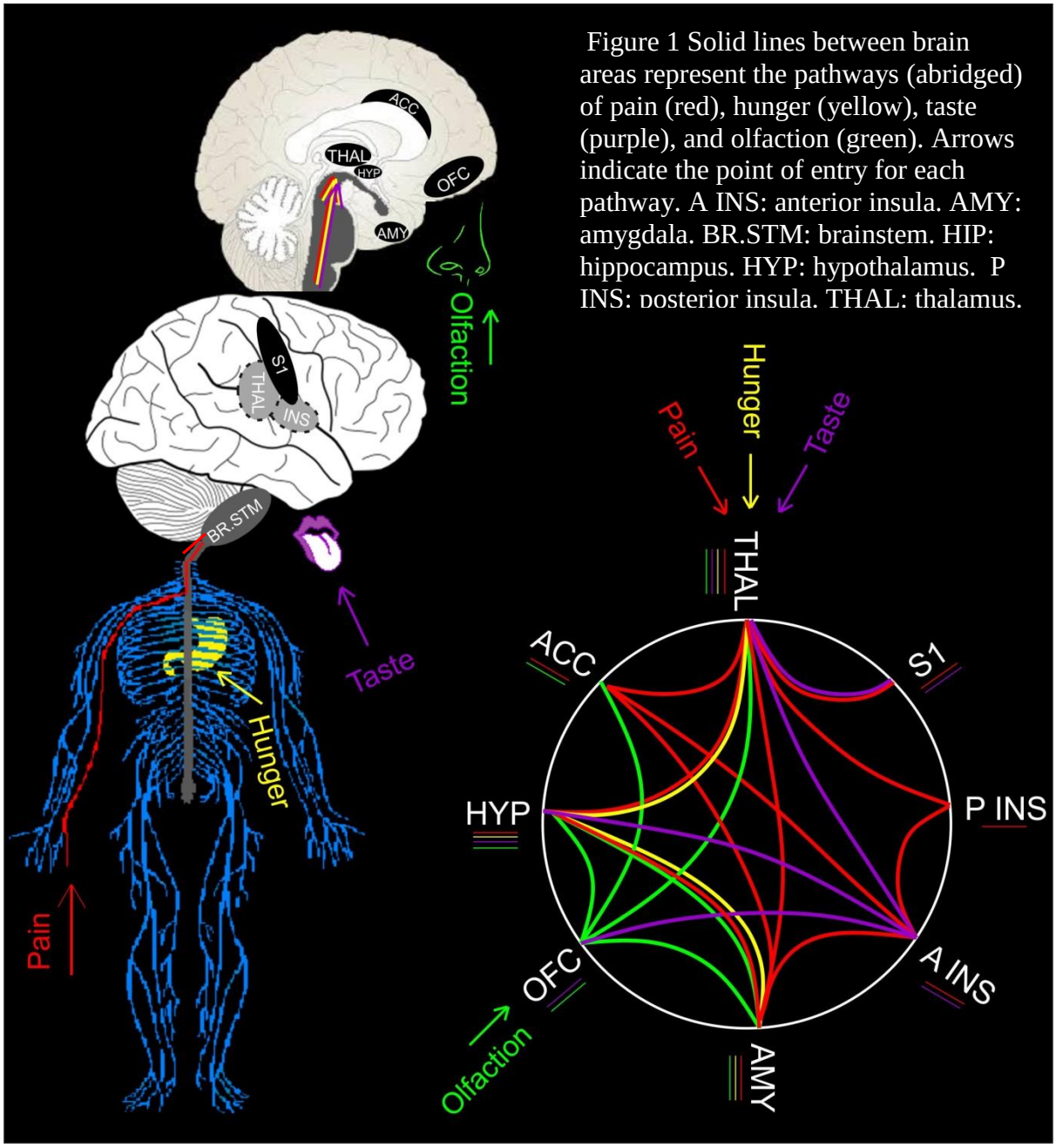

Figure 1 Solid lines between brain areas represent the pathways (abridged) of pain (red), hunger (yellow), taste (purple), and olfaction (green). Arrows indicate the point of entry for each pathway. A INS: anterior insula. AMY: amygdala. BR.STM: brainstem. HIP: hippocampus. HYP: hypothalamus. P INS: posterior insula. THAL: thalamus.

All these areas are phylogenetically ancient (Cho et al. 2013; Egan et al. 2003; Egan et al. 2005; Mashour and Alkire 2013; Nadeau 2008; Parsons et al. 2000), and also underlie other homeostatic drives, such as thirst, fear, hunger for air, and sleep (Brannan et al. 2001; Fischer et al. 2000; Liotti et al. 2001; Sewards and Sewards 2003).

1.7 Literature search results

To investigate interactions between pain, appetite, and food olfaction, the electronic databases Medline, PsycINFO, and PubMed were searched for relevant papers with the following search terms: (Hunger OR Satiety) AND Pain NOT Gastroparesis NOT Dyspepsia NOT Dyspnoea NOT Cancer NOT Diabetes NOT Liver NOT Dyspnea NOT Kidney NOT Vomiting. Articles and book chapters were disregarded if they were written in any other language but English. There were no restrictions with respect to the earliest publishing date. The first search commenced on 03/12/2012 and was completed by 14/12/2012. A further search of the same databases with the same search terms was completed 08/01/2015, and encompassed the time frame 01/12/2012 to 08/01/2015. Another search of PubMed, Medline, and PsycINFO with the search terms (Olfact* AND Pain) NOT Parkinson* NOT Cancer NOT Depress* NOT Spinal NOT Esthesioneuroblastoma NOT Polyposis NOT Stroke was completed 12/01/2015. An additional search for both sets of search terms was completed 29/09/2015, covering the time frame from 01/01/2015 up to and including the search date. Additional papers of interest were identified from the reference lists of retrieved articles. All relevant retrieved papers are detailed in Tables 1A-D.

Table 1A

Authors	Subjects	Primary pain	Fasted	Fed	Primary measure	Primary outcome
Aloisi et al. 2002	Adult rats	Formalin	-	X	Paw licking	Pain ↓ with lemon odour
Aloisi & Carli 1996	Adult rats	Formalin	X	✓	Paw licking	Pain ↓ when food available
Blass et al. 1987	Infant rats	Thermal	-	✓	Paw lift latency	Pain ↓ following sucrose #
Blass et al. 1991	Infant rats	Thermal	-	✓	Paw lift	Pain ↓ following milk #
Blass & Fitzgerald 1988	Infant rats	Thermal	-	✓	Paw lift	Pain ↓ following milk #
Blass & Shide 1994	Infant rats	Thermal	-	✓	Paw lift latency	Pain ↓ following sugars
Chudler & Dong 1983	Adult rats	Surgical	N/A	✓	Body weight	Weight ↓ following surgical pain
Davidson et al. 1992	Adult rats	Thermal	✓	X	Tail flick latency	Pain ↓ following 24 hour fast #
De los Santos-Arteaga 2003	Mice	Chem./thermal	✓	-	Paw lick	Pain ↓ with intermittent fasting #
Dum & Hertz 1984	Adult rats	Thermal	-	*	Paw lick	Pain ↓ when food is expected #
Foo et al. 2009	Adult rats	Formalin	X	✓	Paw movement	Pain rarely interrupted feeding
Foo & Mason 2005	Adult rats	Thermal	X	✓	Paw withdrawal	Pain rarely interrupted feeding
Foo & Mason 2009	Adult rats	Thermal	X	✓	Paw lift / lick	Pain ↓ following palatable food
Gong et al. 2014	Adult rodents	Formalin	X	X	Flinching	Pain ↓ with INT GLP1
Guneli et al. 2010	Adult rats	Surgical/pressure	X	X	Paw withdrawal	Pain ↓ with IP ghrelin
Hua et al. 1991	Adult rats	Thermal	-	X	Pain reflex	Pain ↓ with INT NPY
Ikeda et al. 2014	Adult mice	Formalin	-	X	Paw lick	Pain ↓ with lemon odour
Khasar et al. 2003	Infant rats	Formalin	✓	X	Paw movements	Pain ↑ following 48 hr fast
Kutlu et al. 2003	Adult mice	Thermal	X	X	Paw lick	Pain ↑ with IP leptin
LaGraize et al. 2004	Adult rats	Formalin	✓	✓	Paw movements	Pain ↓ when working for food
Leiphart et al. 2010	Adult rats	Surgical	N/A	✓	Body weight	Weight ↓ in chronic pain model

Li et al. 2002	Adult rats	Thermal	X	X	Paw withdrawal	Pain ↓ with INA NPY # ^
Li et al. 2005	Adult rats	Chem./thermal	X	X	Paw withdrawal	Pain ↓ with ARC NPY ^
Li et al. 2013	Adult rats	Surgical/thermal	-	X	Withdrawal	Pain ↓ with INT leptin
Lim et al. 2009	Adult rats	Surgical/thermal	X	X	Paw withdrawal	Pain ↑ with INT leptin
Malick et al. 2001	Adult rats	Dural stim.	X	✓	Food intake	Intake ↓ after painful stimulation
McGivern & Bernston 1980	Adult rats	Thermal	✓	X	Tail flick	Pain ↓ following a fast #
McGivern et al. 1979	Adult rats	Thermal	✓	X	Tail flick latency	Pain ↓ following a fast #
Nakama-Kitamura 2014	Adult mice	Formalin	-	-	Paw lick	Pain ↓ with sweet food odour
Ramzan et al. 1993	Adult rats	Thermal	X	X	Tail flick latency	Pain ↓ in obese rats
Ren et al. 1997	Infant rats	Chem./thermal	-	✓	Escape latency	Pain ↓ with sucrose + suckling
Segato et al. 1997	Adult rats	Thermal	X	✓	Tail flick	Pain ↓ following sucrose #
Shavit et al. 2005	Adult rats	Surgical	N/A	✓	Body weight	Weight ↑ with good analgesia
Shide & Blass 1989	Infant rats	Thermal	-	✓	Paw lift	Pain ↓ following corn oil/sugar #
Sibilia et al. 2006	Adult rats	Chem./pressure	X	✓	Paw withdrawal	Pain ↓ with ICV ghrelin #
Tian et al. 2011	Adult rats	Chemical	X	X	Withdrawal	Pain ↑ with INT leptin
Wei et al. 2013	Adult mice	Thermal	X	X	Tail withdrawal	Pain ↓ with ICV/IP ghrelin # ^
Wylie & Gentle 1997	Hens	Chemical	✓	✓	Limb movement	Pain ↓ after feeding #
Zeng et al. 2014	Adult mice	Thermal	X	X	Tail withdrawal	Pain ↓ with ICV ghrelin #
Zhou et al. 2014	Adult rats	Surgical/thermal	X	X	Paw withdrawal	Pain ↓ with INT ghrelin ^

ARC = arcuate nucleus, hypothalamus. Chem. = chemical. GLP1 = Glucagon-like peptide-1. ICV = intracerebroventricular drug administration. INA = intra-nucleus accumbens injection. INT = intrathecal injection. IP = intraperitoneal injection. NPY = neuropeptide Y. # = analgesia blocked by opioid antagonist (naloxone or naltrexone). ^ = analgesia blocked by ghrelin/NPY receptor antagonist. * = expected feeding. X = negative; ✓ = positive. ↑ = increased; ↓ = decreased. Where two primary pain methods are indicated, the first was applied to sensitise the area before application of the second. A dash indicates unreported or unclear.

Table 1B

Authors	Subjects	Primary pain	Fasted	Fed	Primary measure	Primary outcome
Akçam 2004	H. infants	HL	X	✓	Crying	Pain ↓ following glucose / fructose
Blass & Hoffmeyer 1991	H. infants	HL	X	✓	Crying	Pain ↓ following sucrose
Blass & Shah 1995	H. infants	HL	X	✓	Crying	Pain ↓ following sucrose
Bucher et al. 1995	P. infants	HL	✓	✓	Crying/BPM	Pain ↓ following sucrose
Bastion et al. 2014	H. adults	CP	-	✓	Self-report	Chocolate liking ↑ following pain
Carbajal et al. 2002	P. infants	Injection	X	✓	Pain score “”	Pain ↓ following glucose
Eggleston et al. 2010	H. adults	CP	✓	X	Pain tolerance	Sweet taste ↑ pain tolerance
Haouari et al. 1995	H. infants	HL	X	✓	Crying	Pain ↓ following sucrose
Lewkowski et al. 2003	H. adults	CP	-	X	Pain tolerance	Pain tolerance ↑ following sucrose |||
Miller et al. 1994	H. children	CP	X	X	Pain threshold	Pain threshold ↑ following sucrose |||
Pollatos et al. 2012	H. adults	Pressure	✓	X	Pain tolerance/threshold	Pain ↑ following fasting
Prescott & Wilkie 2007	H. adults	CP	-	X	Pain tolerance	Pain tolerance ↑ with caramel odour
Wright et al. 2015	H. adults	Laser	✓	✓	Brain activations	Pain activation ↓ when fasted/VFS
Zmarzty et al. 1997	H. adults	CP	✓	✓	Pain rating	Pain rating ↓ following a meal

BPM = beats per minute (heart rate). CP = cold pressor. H. = healthy. HL = heel lance. N = number of subjects. Nutri. ad. = nutrients administered. P. = premature. TL = toe lance. VFS = with visual food stimuli. X = negative; ✓ = positive. ↑ = increased; ↓ = decreased. “” = rated according to behaviour. ||| = nutrient held in the mouth, but not swallowed. Dash indicates unreported or unclear.

Table 1C

Authors	Subjects	Primary pain	Fasted	Fed	Primary measure	Primary outcome
Bosley et al. 2004	PD adults	Chronic	N/A	N/A	Self-report	Appetite impaired by ↑ pain
Drummond 1982	PD adults	Headache	✓	N/A	Self-report	Hunger precipitated headache
Janke & Kozak 2012	PDOB adults	Chronic	-	N/A	Self-report	Hunger/bingeing precipitated by pain
Geha et al. 2014	PD adults	Chronic	X	✓	Self-report	Food hedonicity ratings ↓ in PD
Malick et al. 2001	PD adults	Migraine	N/A	N/A	Self-report	Appetite ↓ coincides with pain onset
Martin & Seneviratne 1997	PD adults	Headache	✓	X	Self-report	Pain precipitated by hunger + NAFF
Michalsen 2010	PD adults	Chronic	✓	X	Self-report	Pain ↓ by fasting (200-500 kcal/day)
Torelli & Manzoni 2010	PD adults	Headache	✓	N/A	Self-report	Hunger precipitates headache
Tosun et al. 2014	SP	Surgery	✓	N/A	Self-report	Fast>12 hours, ↑ pain after surgery

N/A = not applicable. NAFF = negative affect. PD = pain disorder. PDOB = pain disorder with obesity. SP = surgery patient. X = no; ✓ = yes. ↑ = increase; ↓ = decrease. A dash indicates unreported or unclear.

Table 1D

Authors	Subjects	Primary pain	Primary measure	Primary outcome
Abraham & Joseph 1986	ED	Pressure	Pain tolerance	Pain tolerance ↑ after vomiting \\\
Faris et al. 1992	ED	Pressure	Pain detection threshold	Pain detection threshold ↑ in ED
Girdler et al. 1998	ED	Ischemic	Pain tolerance	Pain tolerance ↑ in ED
Lautenbacher et al. 1990	ED	Thermal	Pain threshold	Pain threshold ↑ in ED
Lautenbacher et al. 1991	ED	Thermal	Pain threshold	Pain threshold ↑ in ED
Papezova et al. 2005	ED	Thermal	Pain tolerance	Pain tolerance ↑ in ED
Pauls et al. 1991	ED	Thermal	Pain threshold	Pain threshold ↑ in ED
Raymond et al. 1995	OBED	Pressure	Pain det. threshold	Pain det. threshold ↑ in OBED
Raymond et al. 1999a	ED	Pressure	Pain det. threshold	Pain det. threshold ↑ in bulimic episode
Raymond et al. 1999b	ED	Pressure	Pain det. threshold	Pain det. threshold ↑ in ED
Stein et al. 2003	ED(F)	Ischemic	Pain tolerance	Pain tolerance ↑ in ED(F)
Yamamotova et al. 2009	ED	Thermal	Pain threshold latency	Pain threshold latency ↑ in ED
Geliebter et al. 2012	OB	CP	Eating desire	Eating desire ↓ following pain
Gluck et al. 2004	OBED	CP	Eating desire	Binge eating desire ↑ following pain
Gluck et al. 2014	OBED	CP	Grehlin concentration	Grehlin ↑ following pain
McKendall & Haier 1983	OB	Pressure	Pain threshold	Pain threshold ↓ in OB
Pradalier et al. 1981	OB	Sural nerve	Pain threshold	Pain threshold ↓ in OB
Zahorska-Markiewicz et al. 1983	OB	Electrical	Pain threshold	Pain threshold ↑ in OB

CP = cold pressor. Det. = detection. ED = eating disorder subject (anorexic or bulimic or binge eating disorder). (F) = former eating disorder. OB = obese subject. OBED = obese subject with comorbid binge eating disorder. \\\ = case study with one participant. ↑ = increase; ↓ = decrease.

Two of these studies found that fasting increased pain; Khasar et al (2003), and Pollatos et al (2012). This could be due to the experiment designs employed. Khasar et al deprived rats of food for 48 hours. They did not report the weight loss of the animals during this time, but other studies using 48 hour fasting paradigms have reported total body weight losses of 10.8 % (Kale et al. 2009) to 13.2 % (Li and Wassner 1981). The other studies fasted their subjects from 0 to 24 hours, which would not result in this amount of weight loss. A 48 hour fast also significantly increases glucocorticoid levels and abolishes the diurnal rhythm, suggesting increasing metabolic stress (Toth and Gardiner 2000). Possibly the genuine threat to homeostasis in the Khasar et al study renders their results only loosely comparable to the rest. It is not entirely clear why Pollatos et al found their unusual results. Their participants were fasted for almost 27 hours, a longer stretch than the other studies, but only slightly. However, their participants reported a significantly more negative mood during fasting. Other studies have reported that inducing negative affect increases pain perception (Tang et al. 2008; Villemure and Bushnell 2009; Zelman et al. 1991); possibly the slightly longer than normal fasting time underlies the increase in pain perception, but it seems more likely that the significant increase in negative mood was responsible.

The vast majority of studies found a significant behavioural effect of either feeding or hunger on pain, or vice versa. In studies reporting that pain does not significantly interfere with eating (Aloisi and Carli 1996; Foo et al. 2009; Foo and Mason 2005; LaGraize et al. 2004), feeding has taken precedence over even attending to pain. Similarly, some studies report that appetite or weight is lost throughout pain (Bosley et al. 2004; Chudler and Dong 1983; Geliebter et al. 2012; Leiphart et al. 2010; Malick et al. 2001); here, pain takes precedence over feeding, with food intake restored by effective analgesia (Shavit et al. 2005). A particularly intriguing physiological mechanism that could account for fasting and ingestion analgesia is the gastric branch of the vagal nerve. The vagal nerve is a large structure that begins in the medulla and divides further down to innervate several major areas, including the stomach, large intestine, and colon (Faiz and Moffat 2002; Guyton and Hall 2004). Manipulations of these sub-diaphragmatic branches can affect nociception.

Activation of vagal afferents inhibits perception of several types of pain (Faris et al. 2006; Miao et al. 2003; Sedan et al. 2005). Sectioning the vagal nerve produces

markedly lowered pain thresholds (Chase et al. 1970; Gschossmann et al. 2002; Holtmann et al. 1998; Khasar et al. 1998); artificially electrically stimulating the sub-diaphragmatic vagal nerve inhibits nociception (Chen et al. 2008; Randich and Gebhart 1992). Vagal nerve afferents from the stomach are activated naturally when the stomach starts to fill (Laskiewicz et al. 2003; Schwartz 2000). Fasting may also potentiate nociception, but some of this effect also appears to be due to activation of vagal afferents. Vagal afferent activity increases during fasting (Szekely et al. 2000), and the increase in nociceptive responses during fasting is eliminated when the vagal nerve is cut (Khasar et al. 2003). It is not so simple, then, as activation of vagal afferents always suppressing pain. In line with this theory, different vagal afferents have been shown to produce either pro-nociceptive or anti-nociceptive activity (Gebhart and Randich 1992; Randich and Gebhart 1992). Activation of vagal afferents could feasibly partly explain the anti-nociceptive and pro-nociceptive effects of fasting and feeding in both healthy participants, and those with an eating disorder.

1.8 Interim summary

The relationship between appetite and pain is complex, with many potential moderators and shared brain areas. This thesis is intended to shed more light on the possibility of temporary pain suppression / reduction with appetitive stimuli, and / or manipulations of the homeostatic energy balance. While not directly relevant to chronic pain, due to the transient nature of the nociceptive stimuli and the absence of complicating psychological factors in healthy participants, the studies described hereafter give some indication of how interference with nociceptive processing may be possible.

1.9 Research problems

Thus far, it seems clear that there are several physiological mechanisms that could underlie an interaction between appetite and pain. What remains almost entirely unexplored is the neural basis of interactions between these homeostatic drives. To address this issue requires several stages, each utilising a within-subjects design.

Firstly, we must identify the fasting vs. satiation paradigm necessary to produce reliably dissimilar blood glucose and neural network measurements. This will be investigated simply with short resting state fMRI scans, one after a fast and the other following a meal, both preceded immediately by blood glucose measurements. Having established a suitable fasting and satiation paradigm, the next step is to investigate the effects of appetite on subjective pain perception and neural pain processing. Pain stimuli will be produced using a laser stimulator, which is capable of selectively activating nociceptive Aδ fibres. To further enhance hunger-pain interactions, we will present the pain stimuli concurrently with appetitive food photographs. Neural pain processing will be explored using the well-established method of high-density (128-channel) EEG recordings and source analysis. This will allow for an analysis of the temporal properties of the pain signal, as well as a limited investigation into the spatial sources. Next, to more thoroughly explore brain areas underlying the hunger-pain interaction under fasting and satiated conditions, we will use fMRI to map changes in functional connectivity between regions known to be involved in both pain and appetite. Finally, to confirm that appetitive stimuli across other modalities can also interact with pain processing, we will investigate the effect of food odour on nociception.

We hypothesise that fasting will suppress subjective and neural pain processing, and that visual and olfactory appetitive stimuli may augment such interference.

1.10 Thesis chapters

Chapter 2 describes the general methods and equipment utilised in the following experiments, with particular emphasis on EEG source analysis, functional magnetic resonance imaging (fMRI), and functional connectivity.

Chapter 3 is a resting state fMRI study examining the effect of satiety and glucose on lateralised insula functional connectivity; Chapter 4 is a resting state fMRI exploration of hypothalamic functional connectivity under fasting and satiated conditions, and the relationship between such functional connectivity and deliberate restraint of eating. These studies were carried out to establish whether a relatively short

overnight fast was sufficient to produce robust effects on the resting state functional connectivity of homeostatically important brain areas in healthy participants.

Chapter 5 is an EEG source analysis study, modelling cortical and sub-cortical sources involved in producing laser evoked potentials (LEPs) under conditions of fasting and satiation. Appetitive and non-appetitive visual stimuli were also presented, in order to further explore the effect of immediate contextual manipulation on pain processing when the homeostatic energy balance is manipulated.

Chapter 6 is an event-related fMRI study, investigating the effects of fasting and satiation on brain activations to pain, and pain-evoked connections between brain areas cited frequently in pain and appetite studies. Incorporating both a subtraction and a functional connectivity analysis produces different results and answers different questions. A subtraction analysis can be used to identify distinct brain areas that are activated in response to the pain stimuli. It does not provide any measure of connectivity between the areas. A functional connectivity analysis can identify both connections and the strength of connections between predefined ROIs, even when the strength of connections is equal across conditions. For example, an area responding to pain equally strongly in both fasted and satiated conditions will not be identified by a simple subtraction analysis (Sommer 2002), but can still be a core component of the pain matrix. These analyses provide complementary answers to data interrogation, and it is therefore judicious to run them both.

Chapter 7 is a behavioural study, investigating the effect of food and non-food odours on the perception of strong and weak pain under fasting and satiated conditions.

Chapter 8 is the general discussion. The results of the experimental chapters are summarised, limitations are described, and suggestions for future research are presented.

Chapter 2

General methods

2.1 Questionnaires

Several questionnaires were utilised in the studies reported in this thesis. Since participants were required to complete lengthy screening and pre-experiment setup procedures, validated and short versions of the questionnaires were employed wherever possible.

2.1.1 Food and Activity Diary

The food and activity diary was used for participants to record their meals, snacks, and any physical activities undertaken from 5pm the evening before a study session up until the start of the study session. It served both as a check that participants kept to the study restrictions regarding food / alcohol intake and strenuous exercise on the day before the experiment, and also that participants consumed and exercised roughly similar amounts before both sessions. There is always the possibility that participants would not be truthful when completing their diary, but it did provide a method for identifying participants who genuinely did not remember to stick to the study restrictions.

2.1.2 Profile of Mood States (POMS)

The POMS (McNair et al. 1971) is a 65 item questionnaire measuring mood disturbance, with good reliability (Boyle 1987). It assesses tension, depression, anger, fatigue, confusion, and vigour, and also includes some unrelated dummy items. It has been used successfully to evaluate mood changes over a variety of short interventions (e.g. Tang et al. 2007; Toro-Velasco et al.), including in the context of appetite (Wells et al. 1998). It was included here as other research has shown that mood can have significant effects on both eating behaviour (Gibson 2012; Jauch-Chara and Oltmanns 2014), and pain experience (Jennings et al. 2014; Rainville et al. 2005; Stancak and

Fallon 2013; Stancak et al. 2013; Strobel et al. 2014). As all of the studies in this thesis used a paired design where participants completed the experiments twice, once when fasted and once when satiated, it was important to measure mood profiles and ensure that they did not differ between sessions.

2.1.3 Three Factor Eating Questionnaire – Revised (TFEQR18)

The TFEQR18 (Karlsson et al. 2000) is an 18 item questionnaire that measures cognitive restraint (deliberately restricting food intake to control weight), uncontrolled eating (loss of control over food intake), and emotional eating (eating in response to negative emotional states), and can successfully discriminate between different eating patterns in the general population (de Lauzon et al. 2004). It was included in the studies for post-hoc correlation analysis. No participants were excluded on the basis of their score; potential participants with a physician-diagnosed or self-diagnosed eating disorder were excluded during screening.

2.1.4 Visual analogue scales

Visual analogue scales (VAS) are the standard tools for measuring subjective appetite (Blundell et al. 2009). They are reliable and valid measures, especially within paired designs (Flint et al. 2000; Stubbs et al. 2000). In every experiment 100 mm VAS were used for participants to record their hunger, desire to eat, and prospective consumption (how much food they thought they could eat) at the start of the fasted sessions, and after eating breakfast during the fed sessions.

2.2 Feeding paradigm

Satiation is provoked by accumulating anorectic signalling as food is consumed (Bellisle et al. 2012). It is generally defined as being achieved when someone stops eating of their own accord, not when they stop merely because they have eaten all of the available food. Providing an *ad libitum* meal during the satiating session would have

more closely modelled real life eating behaviour, but due to the critical need for participants to feel full, we decided to make use of a standardised feeding regime whereby participants consumed around 26% of their recommended daily calorie allowance during breakfast. This standardised meal included items such as toast, cornflakes, and cereal bars, and requiring participants to eat all of it ensured that every participant reached a moderate to high level of satiation, as assessed by VAS responses. Had we provided an *ad libitum* meal, some participants would most likely have consumed a small breakfast which would not have allowed them to feel satiated right up to the end of the fed session. Knowing that food intake is being monitored prompts many participants to under eat (Robinson et al. 2014; 2015)

2.3 Equipment

2.3.1 Laser stimulator

An Nd-YAP laser stimulator (Stim1340, El.En.) was used to produce the pain stimuli in Chapters 5 and 6. A spot size of 5 mm and a pulse duration of 3 ms reliably elicited a painful pricking sensation over the stimulated skin. Laser stimuli selectively activate nociceptive fibres (Lockwood et al. 2013; Treede 2003), without introducing the tactile sensations which are inherent when applying mechanical pain stimuli to the skin (Plaghki and Mouraux 2003). The sharp rise-time of laser stimuli enables time-locking of stimuli to evoked responses in the brain, making lasers ideal for use with EEG (Plaghki and Mouraux 2005). Lasers have been used to explore nociception with EEG and fMRI in a large number of studies.

2.3.2 Electrical stimulator

The DS7A general purpose electrical stimulator (Digitimer, Hertfordshire, UK) was used to produce the pain stimuli detailed in Chapter 7. It is capable of delivering transcutaneous nerve stimuli comprised of trains of electrical pulses. Using a train of three stimuli separated by 15 ms produced a subjective sensation of a single painful stimulus, which was scalable over a wide voltage range.

2.3.3 Olfactometer

A custom-built olfactometer (Dancer Design, Wirral, U.K.) was used to deliver the olfactory stimuli described in Chapter 7. The olfactometer has eight separate channels made from fluorinated ethylene propylene (FEP) tubing, connecting the glass bottles containing the odours to a fitting on the participant head piece, which delivered the odours to approximately one cm below the nose. The fitting was comprised of two narrow-diameter FEP tubes, which directed the air flow birhinally. One glass bottle contained only odourless propylene glycol; this was pumped through continually to provide a flow of 'clean air', and was only interrupted by brief pulses of the experimental odours from other bottles. The odours themselves were dissolved in propylene glycol. This configuration allowed the odours to be embedded within the constant flow of clean air, in order to avoid participants sensing changes in air flow associated with odour presentations (Huart et al. 2012). Very similar setups have been utilised successfully in other experiments (Grabenhorst and Rolls 2009; Rolls et al. 2003).

2.4 Biological and physiological measures

2.4.1 Blood glucose sampling

In order to evaluate the effects of glycaemia on experimental results, the Accu-Chek Aviva Blood Glucose Meter system (Roche Diagnostics ltd., UK) was used to measure blood glucose levels before fasted sessions, and approximately 15 minutes after breakfast in fed sessions. This system is available over the counter for self-monitoring, and was selected due to its ease of use and experimentally demonstrated accuracy (Freckmann et al. 2012).

2.4.2 EEG

EEG is a method of recording electrical signals from the brain which are distributed across the scalp. The brain is never at rest; spontaneous electrical signals can be detected even during coma states (Young 2000). The source of the EEG signal is the

activity of groups of neurons distributed throughout the brain. Single neurons generate electrical potentials when sodium, potassium, calcium, and chloride ions are exchanged though the cell membrane. It is not possible to detect the activity of single neurons at the scalp; instead, EEG measures the electrical potentials emitted by large populations of neurons firing in synchrony. It is a non-invasive technique; recordings are made via electrodes placed on the surface of the scalp, with the aid of an electrolyte such as potassium chloride salt dissolved in water. The weak electrical signal detected over the scalp must be massively amplified before recording. The EEG system used in chapter 5 was a 128-channel dense-array net of sponge electrodes (Electrical Geodesics Inc.), covering the entire vertex and back of the head, and much of the face (Figure 2). Signal detected at an individual electrode is not a pure 'readout' of electrical activity at that electrode; it is the difference in voltage between signal at that electrode and a reference electrode (Luck 2005a), usually (but not always) positioned over the vertex.

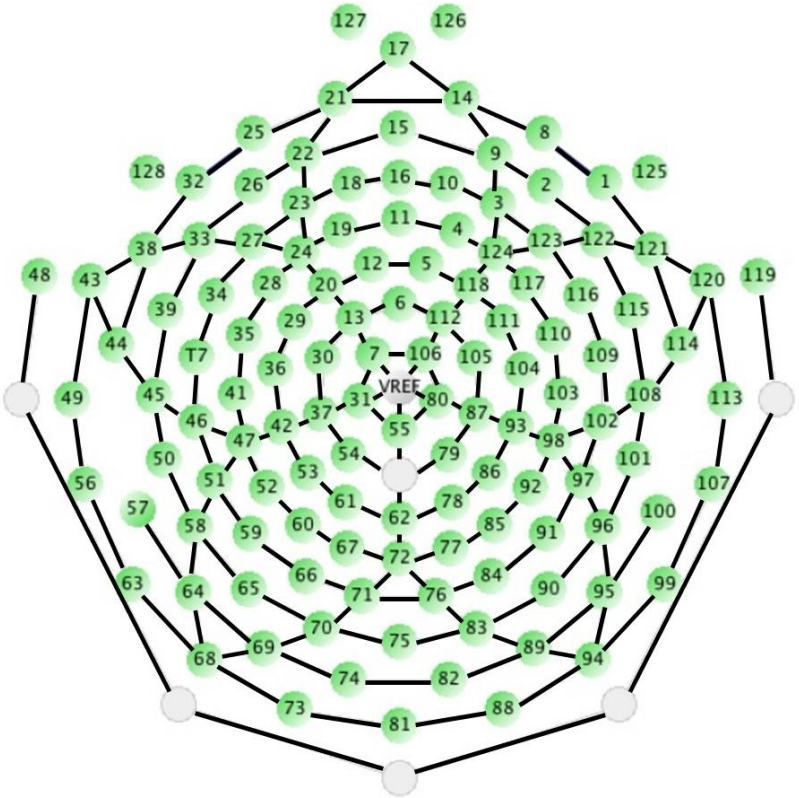

Figure 2

Schematic of the 128 electrode Geodesic sensor net. Electrode 17 is placed between the eyes, approximately 1 cm above the bridge of the nose. Electrodes 126 and 127 are cheek electrodes; electrodes 125 and 128 sit also on the cheeks, ventrally and caudally to 126 and 127. Electrode VREF is more commonly referred to as Cz.

2.4.3 Laser-evoked potentials

An event-related potential (ERP) is an electrical brain response to a stimulus (Luck 2005a), whatever that stimulus might be. ERPs have been studied in a wide variety of contexts, and have been shown to be moderated by many physiological and cognitive manipulations. Of special interest in this thesis are potentials provoked by noxious laser stimuli: laser-evoked potentials (LEPs).

Applying a brief noxious laser pulse to the skin selectively activates Aδ fibres, myelinated axons which transmit fast pain signals to the spinal cord. While other types of painful stimuli such as heat, cold, and trauma can also activate Aδ fibres, laser stimuli do not contaminate the pain signal with additional somatosensory input. They therefore offer a considerable advantage over other methods in terms of stimulus purity.

Nociceptive laser stimuli, first utilised for pain research around 40 years ago (Carmon et al. 1976; Mor and Carmon 1975), generate the characteristic and highly reproducible waveform shown in Figure 3.

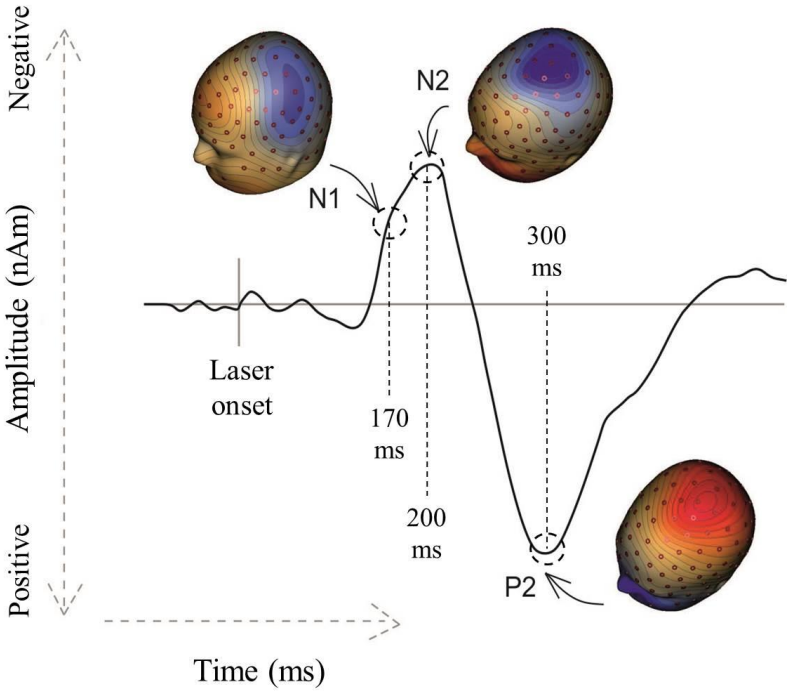

Figure 3 Typical waveform of an LEP derived from electrode Cz (VREF in Figure 2). This waveform is taken from the grand averaged LEPs which were used to generate the source analysis described in chapter 5. The N1 component (the small deflection on the strong negative part of the wave) is best observed over the temporal lobe contralateral to the application site of the laser stimuli. N2 and P2 are most prominent over the vertex. The times in ms quoted for each component are the typical timings reported in LEP studies.

Each of these LEP components (N1, N2, and P2) is susceptible to modulation. The amplitude of N1 is increased in conjunction with increasing pain intensity (Iannetti et al. 2005; Stancak et al. 2012), and appears to be enhanced by more salient noxious stimuli (Iannetti et al. 2008; Ronga et al. 2013). Modulations of N1 can reflect a change in attentional focus (Legrain et al. 2002). The magnitude of N2 has previously been shown to correlate strongly with perceived pain intensity (Iannetti et al. 2005), and is thought to reflect the degree of attentional capture by a salient stimulus (Iannetti et al. 2008). The amplitude of the N2 LEP component is attenuated by distraction (Beydoun et al. 1993; Friederich et al. 2001; García-Larrea et al. 1997; Yamasaki et al. 1999), and enhanced by attention (Legrain et al. 2002). P2 is influenced by affect (Boyle et al. 2008; de Tommaso et al. 2008; 2009; Ring et al. 2013), and represents cognitive processing of nociceptive stimuli (Lee et al. 2009; Mobascher et al. 2009; Wager et al. 2006). Any of these components could be moderated by a competing appetitive drive: N1 and / or N2 by reduced saliency of the nociceptive stimuli; P2 by reduced cognitive processing of nociceptive stimuli.

2.4.4 LEP source analysis

Estimating the brain sources of LEPs requires solving the inverse problem: that activation in the brain could be the result of any of an essentially infinite number of combinations of different populations of neurons firing. There is no unique spatial solution for the voltage measurements across the scalp yields a vast array of data from *n* electrodes x *n* time points, and from each dipole modelling a source, which itself has the parameters of location (x, y, z), orientation angles of the pyramidal cells in the local grey matter, and strength (Grech et al. 2008). Previous research suggests using a minimum of 100 electrodes for reliable source analysis (Michel et al. 2004); after which the localisation accuracy plateaus. The signal itself is distorted by the brain tissue, cerebrospinal fluid (CSF), skull, and skin, which each have different electrical conductivity properties and must be modelled appropriately. In order to narrow down plausible source solutions from the data, it is necessary to impose some constraints on the model. Using results from similar paradigms in neuroimaging research, it is possible to estimate the number of sources contributing to the signal measured at the scalp. This

is usually a low number, with sources added to the model one by one until adding another does not significantly decrease the amount of residual variance.

Low Resolution Electromagnetic Tomography (LORETA; Pascual-Marqui et al. 1994) is a commonly employed method of source localisation. It allows sources to be distributed throughout the whole brain, giving both deep and cortical sources the same chance of being reconstructed, and therefore offering a considerable advantage over other methods which preferentially model sources close to the cortical surface (Grech et al. 2008). As the 'low resolution' part of the LORETA name suggests, the reconstructed images of LORETA are somewhat blurry due to crosstalk between brain areas; an issue that is intensified if sources are spatially close (Liu et al. 2005).

One evolution of the LORETA method is classical LORETA analysis recursively applied (CLARA; Hoechstetter et al. 2010). CLARA (as implemented in BESA 6.0, GmbH) begins with a regularised LORETA image. Iteratively, the image is smoothed and voxels with amplitudes of less than 1% of the maximum amplitude are eliminated from the analysis. This produces a LORETA image that contains the amplitude of each voxel's activity. The resulting image shows activations far more circumscribed than are achievable with other source analysis methods, even when the sources are spatially close. For this reason, CLARA was used to localise the LEPs generated in the EEG study described in Chapter 5.

2.4.5 Considerations of EEG

There are many benefits to using EEG as an investigative tool. It has excellent temporal resolution, on the order of milliseconds, which is far superior to that of fMRI. This high temporal resolution renders EEG invaluable for exploring fast processing. It is also much less expensive than fMRI, and does not induce claustrophobia. It is silent, and does not require the use of the strong magnetic fields which necessitate the exclusion of participants with ferromagnetic implants from fMRI studies.

As noted in section 2.5.2, it is possible to accurately model brain areas contributing to an evoked brain response. However, while EEG is relatively inexpensive in financial terms, this type of modelling is hugely time consuming. It is also not

possible to be sure that activity in every brain region activated has been identified; some deeper parts of the brain are essentially invisible to EEG (Duyn 2012), as are areas curved in such a way that external electrical signals are cancelled out (Nunez et al. 2000; Pacia and Ebersole 1997).

There are other significant disadvantages to EEG. Many trials of stimuli from each experimental condition are required, since noise is generally greater than the signal of interest, and some trials will likely be rejected during the signal cleaning process (Hauk 2013; Luck 2005a; 2005b; 2014). The vast amount of trials required can make experiments very arduous for participants, and induce fatigue and boredom. It is unclear whether trials recorded towards the end of a long session measure the process of interest as reliably as trials recorded earlier on.

Lastly, EEG is susceptible to artefacts caused by eye blinks, pulse, participant movements, jaw clenching, skin potentials, and spontaneous bad electrode contacts. It is possible to remove the voltage potentials caused by oculographic and cardiographic signals using principal components analysis (Berg and Scherg 1994), without creating too much distortion (Scherg et al. 2010), but there is no substitute for collecting clean data in the first place (Luck 2014). While obviously there is nothing to be done to suppress cardiographic signals, it is necessary to task participants with staying very still and minimising their eye blinking before and during stimulus presentation. This requires significant effort from participants.

2.5 MRI

2.5.1 Structural MRI

MRI is a non-invasive technique used to image structures in the brain. The participant lies supine on the scanner bed, which is housed within a large, shielded, super-conducting magnet, cooled by liquid helium. Water in the blood contains hydrogen molecules, which themselves contain protons. These protons align themselves and precess with the direction of the static magnetic field (B0) generated by the scanner, due to their magnetic properties. This is the state of longitudinal magnetisation. During scanning, a radiofrequency (RF) pulse is applied at a specific frequency (the ‘Larmor

frequency'), which causes the protons to be knocked out of their magnetic field alignment into a state of transverse magnetisation. When the pulse is switched off, the protons precess back to alignment with the magnetic field (a process known as longitudinal relaxation). The time taken for the protons to return from transverse magnetisation to 63 % realignment with B0 is the T1 (Pooley 2005). The RF pulse that shifts the protons into transverse magnetisation also causes them to precess in formation. When the pulse is switched off, the precession begins to dephase, due to inhomogeneities in the local magnetic field and transfer of energy between protons. The time taken for the transverse magnetisation to decay by 37 % is the T2 (Pooley 2005). Both percentages are based on an RF pulse inducing a 90° flip angle. The transverse magnetisation induces an electrical current in the RF receivers in the MRI head-coil, which is digitised, filtered to extract frequency and phase information, and stored for later reconstruction (Currie et al. 2013; Pooley 2005).

Grey matter, white matter, and CSF tissue types appear differently on MRI scans, due to the divergent percentages of water and fat. On a T1-weighted scan, white matter is bright, grey matter is grey, and CSF appears dark, with the opposite pattern for T2-weighted scans. Adjusting the time-to-repeat (TR) between RF pulses, and the time-to-echo (TE) from RF pulse to signal detection allows switching from one weighting to the other; short TRs and short TEs produce a T1-weighted scan, long TRs and long TEs produce a T2-weighted scan (Nitz and Reimer 1999). T1 scans are used to image anatomical structures in high detail; T2 scans are more useful for detecting pathology, since CSF is often present in diseased areas due to destruction of grey or white matter.

2.5.2 Functional MRI

No brain 'activation' can be deduced from structural MRI scans; fMRI is used to produce images of brain activity. It is not a direct 'readout' of neuronal activity, though synaptic and blood oxygenation level dependent (BOLD) fMRI signals appear to be closely related (Logothetis et al. 2001; Ogawa et al. 2000; Rees et al. 2000). As a brain area uses oxygen and glucose due to task demands, there is an increase in deoxygenated haemoglobin in the area, which is paramagnetic (distorts the BOLD signal). Vasodilators are released (Attwell and Iadecola 2002), blood flow to the area increases

to keep up with oxygen and glucose demand, and this increase in blood flow leads to a reduction in deoxygenated haemoglobin and an increased level of oxygenated haemoglobin. The heightened oxygenation produces a localised increase in BOLD signal.

The brain is modelled as a 3D space containing around 100,000 3D voxels of equal size, with each voxel containing its time series across the scan. Brain images themselves are comprised of slices which contain a uniform grid of data points. The voxel map of the 3D space therefore contains BOLD signal intensity changes over time, induced by the task or stimulus. The BOLD response is modelled as a convolution of the stimulus paradigm (the 'design matrix') with an HRF to produce a statistical parametric map. The map shows regions where the model accounts for significant variance in the BOLD signal time-course.

To track the origin of the BOLD signal, further gradients (magnetic fields) need to be applied. First, slices are selectively excited in turn with a narrow slice selection gradient (Figure 4, Y co-ordinate). Slices are usually separated by a few mm gap in order to prevent crosstalk between protons bordering the edges of slices, which would reduce contrast (McRobbie et al. 2006). Collecting interleaved slices (e.g. collecting all odd-numbered slices and then all even-numbered slices) is sometimes used to alleviate crosstalk (Hornak 2008; Lipton 2008). When the slice selection gradient is switched off, a second 'phase encoding' gradient (oriented perpendicularly to the slice selection gradient: Figure 4, X co-ordinate) is then applied to the selected slice. The phase encoding gradient encodes spatial information about the signal location. Its strength is variable over space, and the resonance frequencies of the protons along the axis are altered in accordance with the differences in field strength. The spatial resolution of the image is therefore directly proportionate to the number of different intensities of magnetic field applied by the phase encoding gradient (Bushong and Clarke 2015). When it is switched off, a final, uniform frequency encoding gradient is applied (Figure 4, Z co-ordinate). Since the phase differentiation is preserved from the previous gradient application, the frequency encoding gradient enables the 'read out' of frequency information in columns along the X co-ordinate.

Pulse sequences can be used to focus on specific aspects of the image by modifying the RF frequency, the gradient durations and magnitudes, and combining frequency and phase encoding (Narasimhan and Jacobs 2002). Their information results in a matrix, k-space, with each point containing unique phase and frequency information (Blink 2010). Using the reverse Fourier transform, the k-space information can be constructed as a brain image. The brain image can also be deconstructed into k-space (McGowan 2005). Comparison of the BOLD maps obtained under different experimental conditions can be used to identify condition-specific activations of cortical and sub-cortical brain areas.

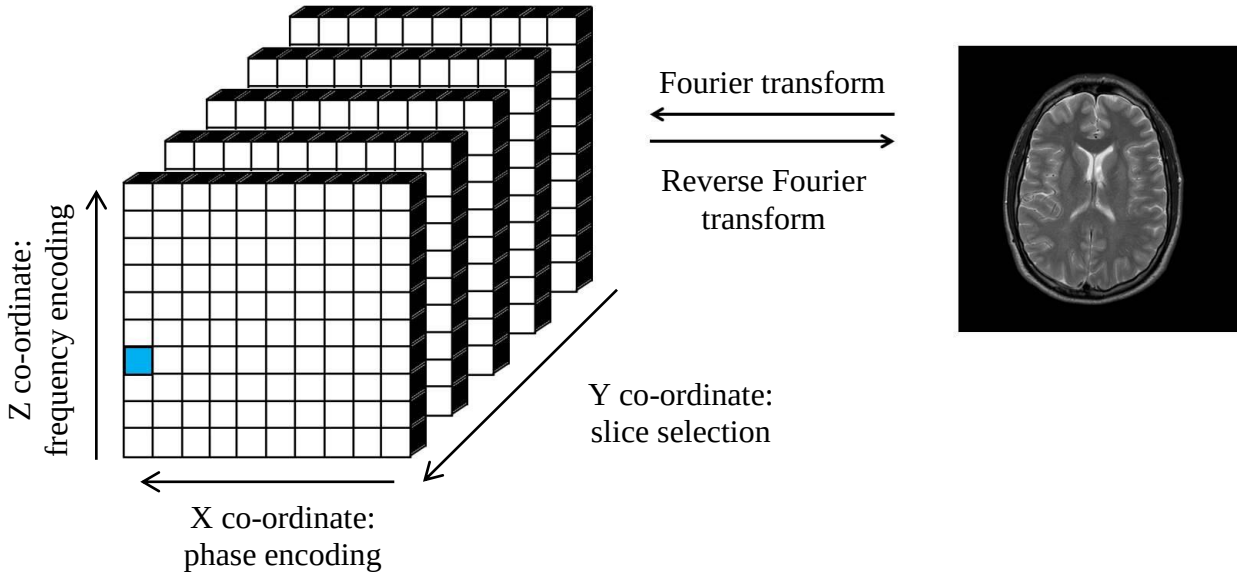

Figure 4 Left: a visual representation of the voxel grid that the k-space matrix covers; right: a T2 brain image. Each cube represents one voxel. Transforming signal from k-space to a brain image requires a reverse Fourier transform. The brain image can be transformed back to the grid via Fourier transform; both hold exactly the same information. The cube selected in blue is located at k-space Y (slice) = 5, X (phase) = 10, Z (frequency) = 4.

2.5.3 Resting-state functional connectivity

As described in section 2.3, the brain is never truly quiet. In the absence of any tasks, separate areas in the brain exhibit spontaneous synchronised activation, which constitute functionally connected networks. Functional connectivity is a measure of statistical association between the time-series of anatomically distinct brain areas (Friston 1994).

It does not imply that the areas are physically directly connected (Friston et al. 1996), and it is not possible to infer the direction of the relationship between the areas, i.e. which area exerts an influence on the other, since this is a correlational technique.

Several networks are reliably observed when participants rest quietly in the scanner. The default mode network (DMN), first described over a decade ago (Raichle et al. 2001), has been extensively studied and found to be abnormal in a huge range of psychiatric and physical diseases. Networks such as the DMN can be identified using independent components analysis (ICA), a data-driven approach that assumes that signal is generated by statistically independent, spatially separated, temporally coherent sources (Calhoun and Adali 2006; Calhoun et al. 2009; Eickhoff and Muller 2015). Components of noise (physiological, head motion, scanner drift etc.) can be visually identified and discarded, and networks of functional connectivity can be compared across different experimental conditions in the same way as a traditional fMRI analysis.

Another approach to analysing resting state data is to use a seed-based method. Defining a region of interest (ROI) which is conceptually related to the topic of interest (for example, a hypothalamus seed in the context of appetite), it is possible to examine correlations between the BOLD signal of the seed and BOLD signal in other areas of the brain. Typical approaches are to look for correlated activity with the seed region across the whole brain, which is an assumption-free method with an entirely data-driven outcome; or to examine functional connectivity between two or more *a priori* defined seed ROIs. Again, the strength of the functional connectivity between the seed and other regions can be compared across experimental conditions. Since the participants included in the following studies were young and healthy, it seemed unlikely that a short overnight fast would induce significant changes in large-scale resting networks. The seed-based approach was therefore utilised for both resting-state studies.

2.5.4 Task-induced functional connectivity

Measures of functional connectivity are generally taken at rest, but have also been used to examine task-induced modulations of connectivity. This analysis method is relatively novel, and so there are few published studies utilising it. Those that do have made use

of simple designs, and include modelling alterations in functional connectivity during reading (Schurz et al. 2015), visual tasks (Goparaju et al. 2014), and working memory (Quide et al. 2013; Sala-Llonch et al. 2012). In Chapter 6, we present the results of an event-related fMRI study in which painful stimuli were applied under fasted and satiated conditions. The first analysis is a standard subtraction in a paired t-test design (pain + fasting > pain +satiety, and *vice versa*). The second analysis is an exploration of pain-induced functional connectivity under fasted and satiated conditions. Since this type of analysis is rare we modelled ours on the recently published work of Vatansever et al (2015), who employed the design most similar to ours and investigated functional connectivity changes induced by a finger movement paradigm. Using the same software toolbox 'Conn' (Whitfield-Gabrieli and Nieto-Castanon 2012) we followed their processing pipeline, using masks derived from brain atlases as ROIs, removing signal from white matter and CSF, adding realignment parameters as first level covariates, and defining individual trials as the onsets of the pain stimuli convolved with a canonical HRF. We then utilised a paired t-test at the group level to examine pain-induced changes in functional connectivity strength in our predefined ROI network under fasted and satiated conditions.

2.5.5 Considerations of fMRI

The main advantage of fMRI over EEG is its vastly superior spatial resolution, on the order of millimetres, though another huge benefit is that there are no sections of brain that are 'invisible' to fMRI. While there can be problems with signal drop-out in areas near to tissue-air interfaces, such as the OFC, changing the slice orientation (Deichmann et al. 2003; Ojemann et al. 1997), optimisation of TE (Domsch et al. 2013), applying a z-shim compensation gradient over the area of drop-out (Cordes et al. 2000; Du et al. 2007), or using a combination of slice-dependent gradient compensation in all three k-space directions, whereby the compensation gradients are optimised for each slice (Rick et al. 2010) can make a marked improvement in signal.

Utilising fMRI is also far less time-consuming than source modelling EEG. The majority of fMRI studies use standardised techniques all the way through from preprocessing (cleaning) the data, to group level results analysis. On the whole, this

eliminates the need to adjust the analysis parameters. It also requires far fewer trials than EEG, due partly to a greatly reduced need for trial rejection.

A significant disadvantage of fMRI is the financial cost of scanning; each scanning session costs several hundred £ more than each EEG recording. Another issue can be participant claustrophobia, induced by the confined space of the scanner and the snug fit of the head coil. Participants must remain still throughout the experiment, which puts additional stress on participants, and restricts the length of experiments. Additionally, the use of the strong magnetic fields necessitates a very careful screening to rule out any potential participants who, knowingly or unknowingly, might have an MRI-unsafe implant or any ferromagnetic debris.

2.6 Summary

EEG and fMRI are complementary neuroimaging methods, each with much to recommend them, and with each compensating for the other's drawbacks satisfactorily. Collecting both is therefore a logical way to obtain a more complete set of cortical observations, providing temporal and spatial acuity, albeit at separate times.

Chapter 3

Differential effects of hunger and satiety on insular cortex functional connectivity

This experiment investigated the effects of manipulations of the homeostatic energy balance on insular cortex functional connectivity.

It is published in the European Journal of Neuroscience (2016), doi: 10.1111/ejn.13182. The format and parts of the content have been altered to match the style of the thesis.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak. Xiaoyun Li and Rebecca Crookall assisted with the data collection. Andrej Stancak and Nicholas Fallon provided training on the data analysis. I analysed the data, interpreted the results, and wrote the manuscript. Xiaoyun Li, Rebecca Crookall, Nicholas Fallon, Timo Giesbrecht, Anna Thomas, Joanne Harrold, Jason Halford, and Andrej Stancak contributed useful comments on the manuscript.

Acknowledgments

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3.1 Abstract

Insula cortex is implicated in eating behaviour, and contains receptor sites for peptides and hormones controlling energy balance. It encompasses multi-functional subregions, which display differential anatomical and functional connectivities with the rest of the brain. The study aimed to analyse the effect of fasting and satiation on the functional connectivity profiles of left and right anterior, middle, and posterior insula. We hypothesised that the profiles would be altered alongside changes in homeostatic energy balance.

Nineteen healthy participants underwent two 7-minute resting state functional magnetic resonance imaging scans, one when fasted and one when satiated. Functional connectivity between the left posterior insula and cerebellum / superior frontal gyrus was stronger during fasting, while functional connectivity between the right middle insula and default mode structures (left and right posterior parietal cortex, cingulate cortex) was stronger during satiation. Differences in blood glucose levels between the scans contributed to increased functional connectivity between the left posterior insula and superior frontal gyrus during fasting, but did not contribute to increased functional connectivity between right middle insula and default mode structures during satiety.

The results suggest that the left posterior insula forms part of a circuit prompting eating when there is an acute deficit in the homeostatic energy balance, whilst right middle insula contributes to the default mode network when satiated. They provide evidence of a lateralised dissociation of insula responses to energy modulations.

3.2 Introduction

Human eating behaviour is determined and influenced by a wide array of internal and external factors. The key motivation is hunger, which (at least in lean individuals) results from a complex interplay between neurochemical compounds, and signalling from homeostatically related brain structures. However, the neural basis of homeostatic networks which drive us to eat requires further elucidation in humans. This is an essential step in understanding dysfunction in appetite regulation that may lead to conditions such as obesity. Insular cortex is a logical region of interest for such a network, since it responds to both orexigenic and anorectic compounds (Schloegl et al. 2011), and is reported as a prominent activation in appetite imaging studies.

The insula is not a homogenous region of cortex. Numerous studies have revealed functional subdivisions, with anterior insula predominantly involved in attentional / emotional processing, and posterior insula in sensorimotor tasks (Cauda et al. 2011; 2012; Chang et al. 2013; Deen et al. 2011; Kelly et al. 2012; Kurth et al. 2010; Stephani et al. 2011). Middle insula is less explored, but is reported to contribute to olfactory / gustatory processing (Kurth et al. 2010) and interoception (Kelly et al. 2012; Simmons et al. 2013). In terms of structure, cytoarchitectonic investigation of insula cortex has revealed three insula subregions; one agranular (anterior), one dysgranular (intermediate), and one granular (posterior) in both primates (Mesulam and Mufson 1985) and humans (Bonthius et al. 2005).

Regarding appetite, anterior insula constitutes part of primary taste cortex and contains neurons responding to a variety of tastes and textures (Rolls 2006; Verhagen et al. 2004). Mid-insula contains neurons coding for taste intensity (Small et al. 2003), is activated in response to visual food stimuli (Schur et al. 2009; Tang et al. 2012), and is implicated in food craving (Pelchat et al. 2004). Posterior insula is activated when people deliberately induce food craving by imagining the taste and smell of food (Siep et al. 2012), in response to visually presented food stimuli (Britton et al. 2006), and to consumption of highly palatable substances (Bohon and Stice 2011).

There is also limited evidence of functional insula lateralisation in the context of appetite and food stimuli, with two recent meta-analyses indicating greater involvement of right insula cortex (Kurth et al. 2010; Tang et al. 2012), while another reported more

involvement of left insula (Kelly et al. 2012). The lack of consensus regarding insula lateralisation and appetite could feasibly be due to the extensive array of experimental designs and stimuli employed between studies.

The current study was designed to investigate appetite-induced functional connectivity changes in anterior, middle, and posterior seeds in left and right insula, using resting state fMRI. Resting state refers to a paradigm whereby participants lie quietly in the scanner without performing any tasks. Functional connectivity measures track temporal correlations in blood oxygen level dependent fluctuations between brain areas, allowing for the identification of coherent brain area networks. We hypothesise that the seeds will exhibit differential patterns of functional connectivity depending on participants' satiation.

3.3 Methods

3.3.1 Participants

Safety screening was carried out by a radiographer, and an additional thorough medical screening was completed by the experimenter. Nineteen healthy Caucasian volunteers (nine male) with a normal body mass index (World Health Organization 2006) took part in this study. The average age of the participants was 24.8 ± 3.8 (mean ± SD). Participants gave their written informed consent and the study was conducted in accordance with the Declaration of Helsinki. Local ethical approval was obtained from the University of Liverpool Research Ethics Committee.

3.3.2 Procedure

Participants attended two sessions, separated by 9.2 days ± 4. On the day before both sessions, participants were reminded not to exercise more than they would normally, and not to eat or drink anything but water after midnight. Compliance was assessed using diary entries and blood glucose testing upon arrival at the imaging facility at 9.30 or 10 am.

Session order was counterbalanced across participants. For the fasted session, they completed the MRI scans after a minimum of a 9.5 hour overnight fast. For the fed session they were given a fixed load breakfast after their overnight fast, and then completed the MRI scans after a short (approximately 20 minutes) delay. The total energy content of the fixed load breakfast was 531 kcal (26.55 % of the recommended daily intake) for females and 670 kcal (26.8 % of the recommended daily intake) for males, and consisted of cornflakes, semi-skimmed milk, toast, margarine, strawberry jam, and orange juice.

Measures of hunger, desire to eat, and prospective consumption (how much food could potentially be eaten) were taken using 100 mm VAS (Stubbs et al. 2000) immediately prior to the MRI scans in both sessions. Blood glucose samples were obtained using a handheld blood glucose monitor (Model: Accu-Chek Aviva, Roche Diagnostics Ltd., West Sussex, UK) immediately prior to the completion of the VAS. The POMS (McNair et al. 1971) was employed to measure participants' mood before the scans in both sessions.

3.3.3 Image acquisition

Scans were undertaken using a whole-body Siemens Trio 3T scanner (Siemens, Erlangen, Germany) with an eight-channel radiofrequency head-coil. Foam padding was used to restrict head movement. A T2-weighted sequence was used to acquire functional resting state images covering the whole brain (32 axial slices), TR = 2000 ms, TE = 30 ms, slice order = interleaved ascending, flip angle = 90°, field of view = 192 mm, slice thickness = 3.5 mm (0.7 cm gap), voxel size at acquisition = 3.0 x 3.0 x 3.5 mm.

In the scanner, participants were asked to relax, close their eyes, and refrain from falling asleep. After the scan they were asked to recall what they were thinking about during the scan, and give a rating between 1 ('not at all') and 7 ('constantly') in response to the question 'How much were you thinking about eating or food?'.

3.3.4 Data analysis

SPM8 (UCL, UK: www.fil.ion.ucl.ac.uk/spm) running on Matlab version R2012a (MathWorks Inc., Natick, MA) was used to preprocess the data. Images were first slice-timing corrected, then realigned to the first slice and unwarped, normalised to the template echo planar imaging (EPI) image, and smoothed using an 8 mm full width half maximum Gaussian kernel.

Preprocessed images were imported to the functional connectivity toolbox 'Conn' v.13 (www.nitrc.org/projects/conn; Whitfield-Gabrieli and Nieto-Castanon 2012). The grey matter, white matter, and cerebrospinal fluid masks were produced by segmenting SPM8's template EPI image using the segmentation routine in SPM8. No 'partition clean-up' was performed, and the ICBM European brains template was used for affine regularisation.

Seed masks were produced using MarsBaR (http://marsbar.sourceforge.net/; Brett et al. 2002). A study on functional differentiation within human insula (Cauda et al. 2011) was used to define left and right anterior, middle (called 'transitional zone' in the original paper), and posterior insula seeds for the current analysis. Each seed is comprised of multiple 5 mm^3 clusters, shown in Figure 5 and detailed in Table 2. Grey matter, white matter, cerebrospinal fluid, and seed masks were resliced to match the image dimensions of the preprocessed functional images.

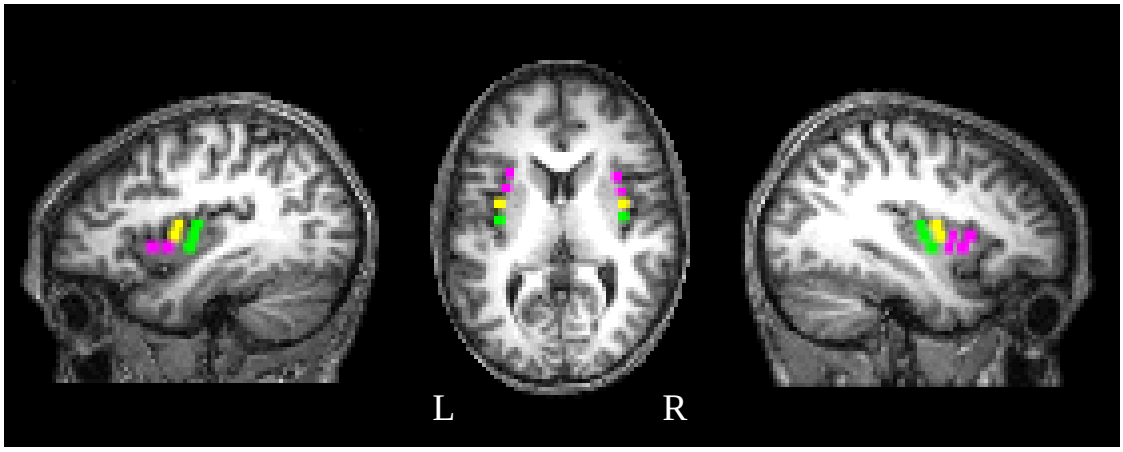

Figure 5 Axial (middle) and sagittal (left and right) views of a randomly selected participant's T1 scan, with anterior insula (magenta), middle insula (yellow), and posterior insula (green) seeds overlaid. L = left; R = right.

		Left				Right			
Seed	**Clusters**	**X**	**Y**	**Z**	**Cluster k**	**X**	**Y**	**Z**	**Cluster k**
Ant. Ins.	1	-34.5	12.5	-2.5	-	34.5	12.5	-2.5	-
	2	-36.5	4.5	-3	-	38.5	5.5	-2.5	-
	3	-30.5	18.5	5.5	-	34.5	16.5	5.5	-
	4	-32.5	9	5	-	36.5	7	5	-
	5	-30.5	9	11.5	936	32.5	9	11.5	864
Mid. Ins.	1	-36.5	-.05	4.5	-	38.5	-0.5	4.5	-
	2	-34.5	-3	11	432	34.5	-3	11	432
Post. Ins.	1	-36.5	-7.5	-3.5	-	36.5	-4.5	-3	-
	2	-36.5	-10	4	-	38.5	-8	4	-
	3	-34.5	-13	10	648	34.5	-11	10.5	648

Table 2 Co-ordinates of the individual 5 mm^3 clusters defined by Cauda et al (2011), grouped into left or right anterior insula seed (Ant. Ins.), middle insula seed (Mid. Ins.), or posterior insula seed (Post. Ins.). Co-ordinates were given in Talairach space in the original paper; those presented here have been transformed into MNI space using the Matlab script 'tal2mni' (Brett 2001), and rounded up or down to the nearest 0.5 mm. Cluster k refers to the number of voxels overall within left or right anterior, middle, and posterior insula seeds.

Individual participants' realignment parameter files were added as first level covariates. Data were initially bandpass filtered from 0.008-0.09 Hz in order to remove noise and low frequency drift. Finally, signal from white matter and CSF was entered as confounds and removed using linear regression.

Remaining data were entered into first-level analysis in a paired t-test design. Individual seed-to-voxel connectivity maps for each seed and each participant were generated separately for the fasted and fed sessions. For second-level analysis, one-sided t-tests were employed to examine changes in seed-to-voxel connectivity between sessions. Seed connectivities were analysed concurrently, and the results were

thresholded at $p < .05$ FWE corrected in order to account for multiple comparisons. Glucose scores were entered as a second-level covariate, expressed as a function of Δ (difference) between the sessions.

Figures 5 and 6 were produced using xjView v.8.12 (www.alivelearn.net/xjview8/) and MRIcron (www.nitrc.org/projects/mricron; Rorden et al. 2007).

3.4 Results

3.4.1 Self-report measures and glycaemia

In comparison to the fed session, during the fasted session participants reported feeling significantly more hungry (mean increase of 53.46 ± 18.5 on the VAS; $t(18) = 12.53$, $p < .001$) before the scan, and more distracted by thoughts of food or eating ($t(18) = 4.31$, $p < .001$) during the scan. Participants also recorded significantly lower blood glucose levels before the fasted scan (mean fasted reading = 5.07 mmol/L ± .35; mean fed reading = 6.79 mmol/L ± .72; $t(18) = -10.58$, $p < .001$). The POMS scores were not significantly different between sessions ($t(18) = -1.54$, $p > .05$).

3.4.2 Functional connectivity

When participants were fasted, we observed enhanced functional connectivity between left posterior insula and right superior frontal gyrus (SFG), and between left posterior insula and left cerebellum (Figure 6A). When participants were fed, they exhibited enhanced functional connectivity between right mid-insula and left inferior parietal cortex (IPC), right IPC, and cingulate cortex (Figure 6B). The MNI co-ordinates and t-scores are shown in Table 3.

Fasted > Fed

Seed	**Cluster**	**Cluster MNI x, y, z (mm)**	**K**	**T (1, 18)**	**P-FWE cluster**
LPI	SFG	16 50 50	142	5.72	0.03
	Cerebellum	-26 -80 -38	208	4.72	0.004

Fed > Fasted

Seed	**Cluster**	**Cluster MNI x, y, z (mm)**	**K**	**T (1,18)**	**P-FWE cluster**
RMI	Cingulate	2 -38 38	206	5.02	0.004
	L IPC	-40 -54 48	236	5.05	0.002
	R IPC	38 -68 42	302	6.85	0.0003

Table 3 MNI = Montreal Neurological Institute. K = cluster size (voxels). FWE = family-wise error. LPI = left posterior insula. RMI = right middle insula. SFG = superior frontal gyrus. L IPC = left inferior parietal cortex. R IPC = right inferior parietal cortex. MNI co-ordinates refer to the peak activated voxels.

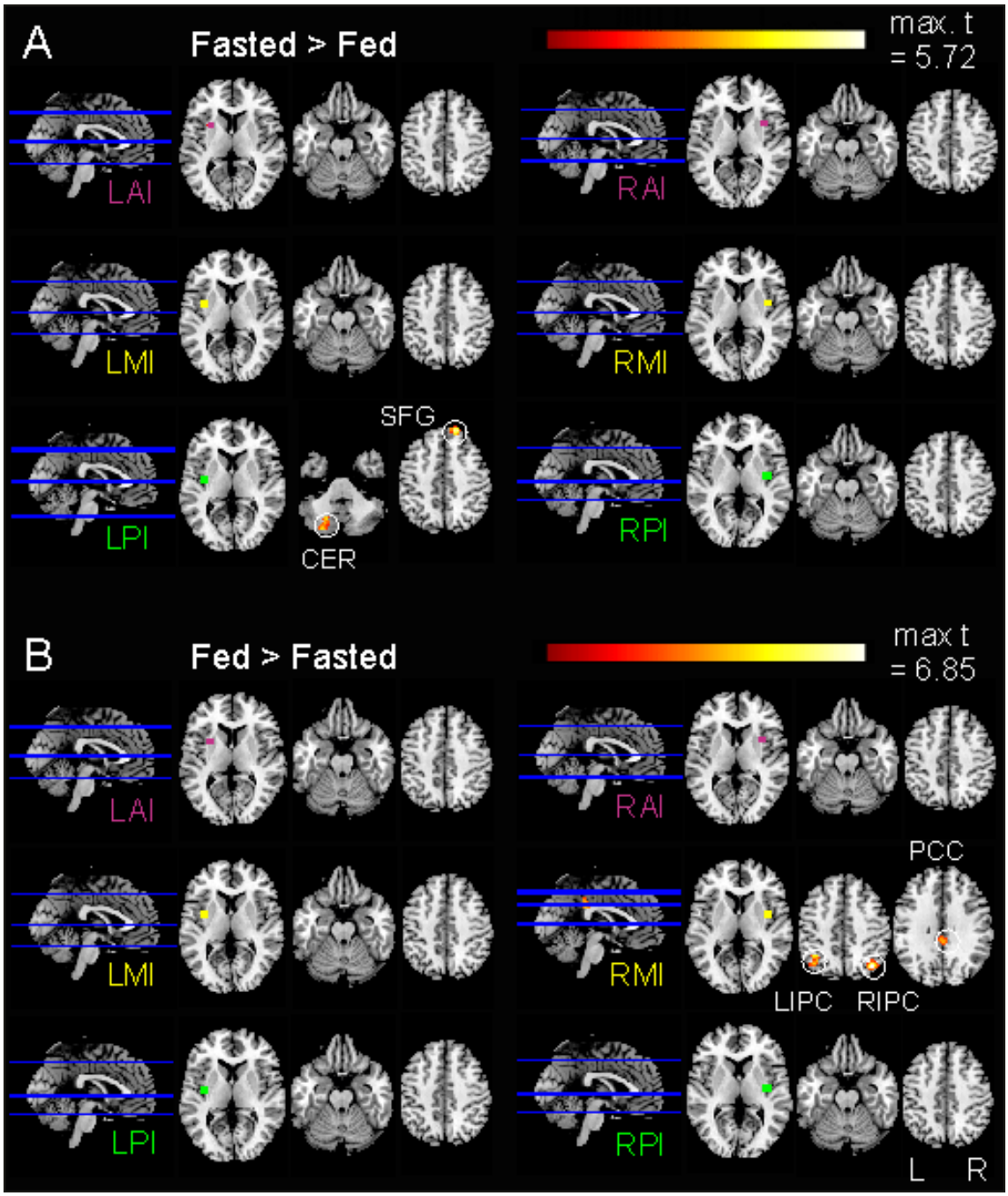

Figure 6 Slice locations (horizontal blue slices), insula seeds, and connectivity maps superimposed over the brain-extracted template in MRIcro. The seeds shown in Figure 5 are displayed again here, with magenta for anterior insula, yellow for middle insula, and green for posterior insula. The activations circled show altered functional connectivity with their respective seeds under conditions of fasting and satiety. Panel A shows seeds and functional connectivities for the contrast fasted > fed; panel B shows seeds and functional connectivities for the contrast fed > fasted. L = left; R = right. AI = anterior insula; MI = mid insula; PI = posterior insula. CER = cerebellum. SFG = superior frontal gyrus. IPC = inferior parietal cortex. PCC = cingulate cortex.

3.4.3 Covariates

The enhanced functional connectivities between left posterior insula and SFG, and right middle insula and posterior cingulate cortex (PCC), disappeared when glycaemia was added as a second level covariate. Correlations between Δ functional connectivities and Δ glycaemia for the connectivities between left posterior insula and SFG, and right middle insula and cingulate cortex are shown in Figure 7, the top graphs of panels A and B respectively.

In order to ascertain whether this finding indicates that the augmented connectivities were at least partially related to differences in blood glucose levels across sessions, or whether the glycaemia measure is just a proxy for a measure of appetite, correlations between functional connectivities and blood glucose were computed separately for the fasted and fed conditions. These results are shown in the middle and lower graphs of Figure 7. Only one is significant: the correlation between left posterior insula and SFG connectivity and blood glucose during the fasted condition (panel A, middle graph). Therefore only left posterior insula to SFG functional connectivity is truly related to blood glucose levels; the right middle insula to cingulate cortex connectivity changes are influenced by satiety status.

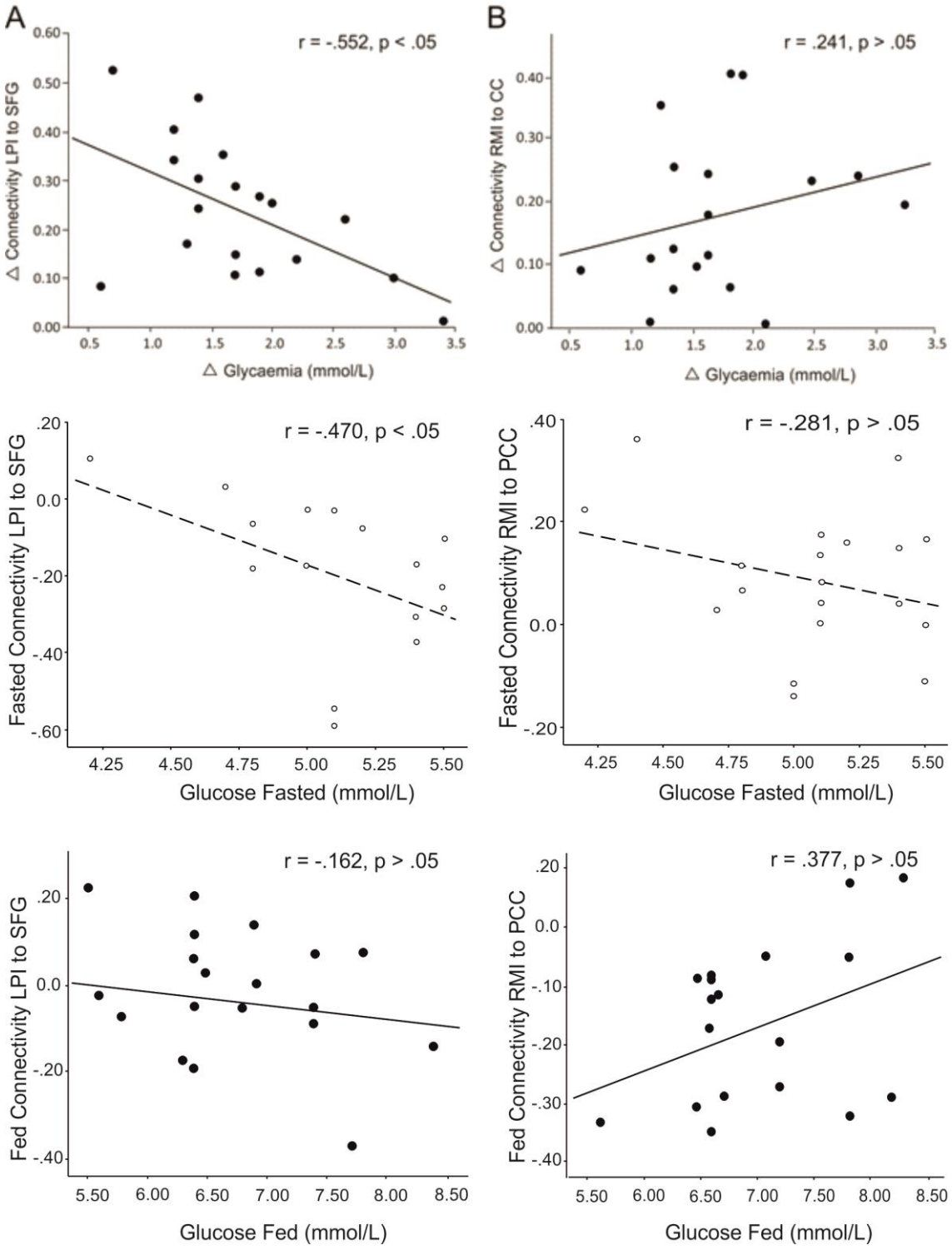

Figure 7 Δ (difference) correlations between functional connectivities and the glycaemia covariate. Δ refers to the difference in connectivity and glycaemia scores between the fasted and fed conditions. Panel A: top graph y axis is Δ connectivity between sessions from left posterior insula (LPI) to SFG correlated with Δ glucose; middle graph is the correlation between LPI-SFG functional connectivity and blood glucose in the fasted session; lower graph is the correlation between LPI-SFG functional connectivity and blood glucose in the fed session. Panel B: y axis is Δ connectivity between sessions from right middle insula (RMI) to posterior cingulate cortex (PCC) correlated with Δ blood glucose; middle graph is RMI-PCC connectivity

correlated with blood glucose in the fasted session; lower graph is RMI-PCC connectivity correlated with blood glucose in the fed session.

3.5 Discussion

During the fasted session, left posterior insula displayed enhanced connectivity with cerebellum and right SFG. Posterior insula has been shown to be activated during hunger (Tataranni et al. 1999), deliberately induced food craving (Siep et al. 2012), in response to a preferred food odour (Bragulat et al. 2010), and on receiving an appetitive drink (Bohon and Stice 2011), and represents a wide range of homeostatically-related sensations in addition to hunger, such as thirst, dyspnoea, and pain (Craig 2002; 2003a).

Cerebellum contains a dense population of leptin receptors (Burguera et al. 2000; Couce et al. 1997; Harvey 2003; 2007; London et al. 2011), is activated during hunger (Tataranni et al. 1999), and exhibits significant decreases in activation after feeding (Del Parigi et al. 2002; Gautier et al. 2000; 2001; Haase et al. 2011). The enhanced functional connectivity seen between left posterior insula and cerebellum suggests a homeostatic circuit motivating food ingestion after fasting.

The SFG is part of a frontal network that represents the motivational or reward value of different foods (Hare et al. 2009; Killgore et al. 2003). It has been frequently theorised to be involved in inhibiting approaches to food (Batterink et al. 2010; DelParigi et al. 2007; Gautier et al. 2000; McCaffery et al. 2009; Tataranni et al. 1999), and is also activated in response to appetitive stimuli when participants are fasted (Burger and Stice 2011; Malik et al. 2011; Martens et al. 2013). It therefore appears to serve a dual purpose, motivating either approach to food or restraint from eating, according to the homeostatic energy balance. While we did not present any appetitive stimuli, participants nevertheless reported experiencing significantly more thoughts about food or eating during the fasted session scan, raising the possibility that their thoughts acted as appetitive stimuli.

The enhanced connectivity between posterior insula and SFG during the fasted session was abolished when glycaemia was added as a covariate. During the fasted session posterior insula-SFG connectivity was significantly negatively correlated with

blood glucose, providing support for the theory that it is glycaemia and not merely hunger that had an effect on the functional connectivity. Taken together, the results suggest that fasting-induced alterations in functional connectivity appear to be related to alleviating an acute homeostatic energy deficit.

During the fed session, right mid-insula was more strongly functionally connected to left and right IPC, and to PCC. Many studies have cited left IPC, right IPC, and PCC as being part of the default mode network (DMN: Fox et al. 2005; Fransson 2005; Greicius et al. 2003; Laird et al. 2009; McFadden et al. 2013; McKiernan et al. 2003; Vincent et al. 2006). The DMN activations represent the 'resting state' of the brain, deactivating in response to task demands. It appears to underlie self-referential and memory-related processes, with memory consolidation (Miall and Robertson 2006), remembering and thinking about the future (Buckner and Carroll 2007), and mind-wandering (Mason et al. 2007) being among the observed functions of the DMN.

Other functional connectivity studies report resting state connectivity between insula and areas of DMN (Li et al. 2012b; Liang et al. 2013; Taylor et al. 2009; Zou et al. 2009). While research has demonstrated the involvement of mid-insula in feeding behaviour (Li et al. 2012a; Small et al. 2001), and some association between DMN activity and obesity (Kullmann et al. 2012; McFadden et al. 2013; Tregellas et al. 2011), it is likely in the context of the current study that its enhanced functional connectivity with DMN areas has more to do with introspective processes, since the homeostatic energy balance had already been restored. That glycaemia is not significantly correlated with changes in middle insula functional connectivity adds weight to this notion. Mid-insula has previously been cited as a region strongly associated with interoception (Kelly et al. 2012; Simmons et al. 2013), and in the current study with healthy participants, the enhanced mid-insula to default mode structures connectivity was accompanied by a reduction in self-reported thinking about food and eating.

3.5.1 Limitations

Blood glucose samples were obtained using a handheld blood glucose monitor, and as such, serum samples are not available for further analysis. Blood glucose sampling was included initially as a crude verification of clear differences between the fed and fasted states. Ultimately it proved a much more important measure than originally anticipated, and taking a comprehensive profile of blood serum may well have provided additional interesting results. However, some inferences regarding commonly studied appetite-related hormones can be drawn on the basis of other research using similar designs and healthy weight participants.

Alterations in free leptin levels are associated with activation changes in insular cortex and other homeostasis-related brain areas (Farr et al. 2014; Olivia et al. 2014). However, leptin has previously been shown to remain at a stable level for at least 2 hours after a test meal (Carlson et al. 2009; Korbonits et al. 1997), and only begins to fall significantly after around 16 hours of fasting. Both these timing parameters exceed those utilised in the present study (minimum 8 hour fast; scanned approximately 20 minutes after breakfast), and therefore it seems unlikely that changes in leptin were a factor in our results.

Feeding has a more acute effect on ghrelin release; the levels appear to be significantly decreased approximately 20 – 30 minutes after a test meal (Carlson et al. 2009; Carroll et al. 2007). Insulin concentration is significantly increased from baseline fasting levels approximately 15 minutes after a test meal, and does not begin to drop noticeably for at least 30 minutes (Carlson et al. 2009; Carroll et al. 2007). Both compounds are associated with the modulation of insula cortex activation (Berthoud 2011; Li et al. 2012a; Malik et al. 2008; Schloegl et al. 2011; Wang et al. 2008a), and both exhibit peak changes in concentration at around the length of time after a meal that our participants were being scanned. It is therefore likely that there are unaccounted for additional hormonal factors influencing or being influenced by changes in these functional connectivities.

3.5.2 Conclusion

Insula functional connectivity patterns are altered by changes in the homeostatic energy balance, with left posterior insula connectivity part of a circuit motivating feeding behaviour. Further research could examine insula connectivity differences between lean and obese participants; possibly a failure to decrease functional connectivity between posterior insula and SFG would be found in obesity. If so, this could represent a new target for interventions.

Chapter 4

The Effect of Appetite on Lateralised Hypothalamic Functional Connectivity

This experiment investigated the effects of manipulations of the homeostatic energy balance on hypothalamic functional connectivity, and the relationship between resting state hypothalamic connectivity, eating habits, and BMI.

It was under review in PLoS One at the time of this thesis submission, but at a reviewer's request its contents have since been incorporated into the European Journal of Neuroscience article presented in Chapter 3.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak. Xiaoyun Li assisted with the data collection. Andrej Stancak and Nicholas Fallon provided training on the data analysis. I analysed the data, interpreted the results, and wrote the manuscript. Xiaoyun Li, Nicholas Fallon, Timo Giesbrecht, Anna Thomas, Joanne Harrold, Jason Halford, and Andrej Stancak contributed useful comments on the manuscript.

Acknowledgments

We gratefully acknowledge Bill Bimson and Val Adams for the liberal sharing of their excellent technical expertise, and their vital support with data collection. We are also thankful to Rebecca Crookall for all the time and indispensable assistance she dedicated to this study. Finally, we are indebted to Dr Keith Sudheimer, who generously shared his hypothalamus mask with our group.

4.1 Abstract

As the obesity epidemic continues, there is no definitive explanation for why some people become overweight while others do not. It is possible that differences in neural connectivity might be a risk factor for becoming overweight.

Hypothalamus is the chief brain region for control of eating behaviour, and is anatomically and functionally connected with a number of homeostatic brain regions. To shed light on the role of hypothalamus in controlling brain activity during fasting and satiation, we took left and right hypothalamus as seeds and, using resting-state fMRI, mapped changes in their functional connectivity induced by alterations in the homeostatic energy balance. Glycaemia, mood, hunger, and TFEQR18 data were also collected. Eighteen healthy weight people (9 male) participated.

Following satiety, there was enhanced functional connectivity between right hypothalamus and superior parietal cortex. During fasting we observed enhanced functional connectivity between left hypothalamus and right inferior frontal gyrus, which was negatively correlated with body mass index. Further, a significant proportion of the variance in BMI could be accounted for by the fasting functional connectivity between left hypothalamus and inferior frontal gyrus.

These areas appear to form a homeostatic energy balance network related to cognitive restraint of eating; preventing overeating when energy is depleted, and ending feeding or transferring attention away from food upon satiation. Further research is necessary to explore the function of this homeostatic mechanism and its relationship to the risk of being overweight.

4.2 Introduction

A major proportion of adults in the western world are overweight, with some researchers postulating an alarming increase in obesity rates over the coming decades (Bibbins-Domingo et al. 2007; Finkelstein et al. 2012; Wang et al. 2008b). Understanding risk factors for becoming overweight is therefore vital for improving public health and controlling health spending. We live in an 'obesogenic environment' (Berthoud 2012; Chaput et al. 2011; Lake and Townshend 2006). Some people can resist appetitive food cues, while other people have difficulty doing so (Loeber et al. 2013; Ouwehand and Papies 2010); possibly, differences in neural connectivity have a role to play (Passamonti et al. 2009). An examination of changes in functional brain connectivity in response to appetite manipulations could help to shed some light on this question.

Homeostatic energy balance is maintained by a variety of brain structures, most notably the hypothalamus (Frank et al. 2012; Schloegl et al. 2011; Schneeberger et al. 2014). Hypothalamus is a target for a variety of neurochemical compounds, both anorectic and orexigenic (Kageyama et al. 2012; Little et al. 2014; Menyhert et al. 2006; Merchenthaler et al. 2010; Morton 2007; Sakurai 2003; Schloegl et al. 2011; Valassi et al. 2008; Williams et al. 2000). It plays a crucial role in food intake (Grill and Kaplan 2002; Palkovits 2003), and is extensively involved in homeostatic metabolic regulation (Carey et al. 2013; Coll and Yeo 2013; Kilpatrick et al. 2014; Zhang et al. 2013; Zhou and Rui 2013). Eating (Thomas et al. 2015), and glucose (Flanagan et al. 2012; Little et al. 2014; Matsuda et al. 1999; Page et al. 2013; Smeets et al. 2005; 2007) or insulin administration (Kullmann et al. 2012) exert significant suppressive effects on the BOLD signal within the hypothalamus.

Hypothalamus is physically connected to other areas involved in maintaining the homeostatic energy balance: cerebellum (Zhu and Wang 2008), brainstem (Berthoud 2012; Grill 2006; Purnell et al. 2014; Suzuki et al. 2012), insula (Lemaire et al. 2011), thalamus (Colavito et al. 2014; Keifer Jr et al. 2015; Lemaire et al. 2011), and prefrontal cortex (Barbas et al. 2003; Groenewegen and Uylings 2000; Rempel-Clower and Barbas 1998). Brainstem receives direct projections from the gastrointestinal tract (Browning and Travagli 2010; Suzuki et al. 2012), and projects directly on to hypothalamus

(Blouet and Schwartz 2010); reduced integrity of white matter tracts in the brainstem is associated with increased body mass index (Verstynen et al. 2012). Cerebellum (Tataranni et al. 1999) and insula (Bragulat et al. 2010; Hinton et al. 2004; Li et al. 2012a; Malik et al. 2011; Tataranni et al. 1999; Teh et al. 2010) show increased activation during hunger, as does thalamus (Li et al. 2012a; Page et al. 2013; Teh et al. 2010). Satiation prompts an increase in prefrontal cortex activation (Thomas et al. 2015), as does purposefully suppressing food desire (Giuliani et al. 2014; Hollmann et al. 2012; Yoshikawa et al. 2014) and successfully dieting (DelParigi et al. 2007), while reduced grey matter volume in prefrontal cortex is related to future increase in BMI (Yokum et al. 2012). These results suggest a top-down modulation of eating by prefrontal cortex.

Most studies do not distinguish between right and left hypothalamus, but some lateralised results have been reported. Left hypothalamus is sometimes found to be related to affect (Agroskin et al. 2014; Cerqueira et al. 2008; Kulkarni et al. 2005), though it has been shown to be smaller in anorexia patients (Titova et al. 2013), and modulated by leptin (Rosenbaum et al. 2008). Right hypothalamus seems more related to homeostatic appetitive processing, demonstrating activation to visual food stimuli (Rosenbaum et al. 2008), especially those depicting food with a high energy content (van der Laan et al. 2011), or following weight loss in obesity (Hinkle et al. 2013). It is at least partially responsible for the anorectic response to acute nicotine administration (Kroemer et al. 2013), and its functional connectivity is modulated by leptin (Hinkle et al. 2013).

The current study was designed to investigate appetite-induced resting state functional connectivity changes in the hypothalamus using fMRI. We hypothesise that right and left hypothalamus will exhibit differential patterns of functional connectivity depending on participants' satiation, with alterations in connectivity reflecting engagement of areas crucial for maintaining homeostatic energy balance.

4.3 Methods

4.3.1 Participants and procedure

Nineteen healthy participants (nine male) aged 24.8 ± 3.8 (mean ± SD) volunteered for this study. One was later excluded, due to their BMI falling outside the predetermined healthy range for the study. Participants gave their written informed consent and the study was conducted in accordance with the Declaration of Helsinki. Local ethical approval was obtained from the University of Liverpool Research Ethics Committee (RETH 000525).

Participants were screened for MRI contraindications by a radiologist. They then completed a thorough medical screening with the experimenter, and filled out the TFEQR18 (Karlsson et al. 2000).

Participants attended two sessions. For the fasted session, they were scanned after a minimum of a 9.5 hour overnight fast. For the fed session they were given a fixed load breakfast after an overnight fast, and then completed the MR scans after approximately 15 minutes. Session order was counterbalanced across participants. The total energy content of the breakfast was 531 kcal (26.55 % of the recommended daily allowance) for females, and 670 kcal (26.8 % of the recommended daily allowance) for males. The macronutrient profile was approximately 11 % fat, 79 % carbohydrate, and 10 % protein (as calculated by www.eatracker.ca/recipe_analyzer.aspx).

Immediately prior to the scans, blood glucose samples were obtained using a handheld blood glucose monitor (Model: Accu-Chek Aviva, Roche Diagnostics Ltd., West Sussex, UK). The POMS (McNair et al. 1971) was employed to measure participants' mood before the scans in both sessions, and hunger was measured with 100 mm VAS. In the scanner, participants were asked to relax with their eyes closed, and refrain from falling asleep.

After the scan they were asked to recall what they were thinking about during the scan, and give a rating between 1 ('not at all') and 7 ('constantly') in response to the question 'How much were you thinking about eating or food?'. No participants reported falling asleep.

4.3.2 Image acquisition and data analysis

Scans were acquired on a whole-body Siemens Trio 3T scanner (Siemens, Erlangen, Germany) with an eight-channel radiofrequency head-coil. A T2-weighted sequence was used to obtain functional resting state images covering the whole brain (32 axial slices), TR = 2000 ms, TE = 30 ms, slice order = interleaved ascending, flip angle = 90°, field of view = 192 mm, slice thickness = 3.5 mm (0.7 cm gap), voxel size at acquisition = 3.0 x 3.0 x 3.5 mm.

SPM8 (UCL, UK: www.fil.ion.ucl.ac.uk/spm) running on Matlab version R2012a (MathWorks Inc., Natick, MA) was used for data preprocessing. Images were slice-timing corrected, realigned and unwarped, normalised to the template EPI image, and smoothed with an 8 mm full width half maximum Gaussian kernel.

These preprocessed images were entered into the functional connectivity toolbox 'Conn' v.13 (www.nitrc.org/projects/conn; Whitfield-Gabrieli and Nieto-Castanon 2012). As the hypothalamus seed mask we used a tracing around bilateral hypothalamus (as defined by the AAL atlas; Tzourio-Mazoyer et al. 2002) produced by Sudheimer et al (2015). The mask was resliced to match our functional image dimensions using the 'Coregister' function in SPM, and then entered into Conn in the form of separate left and right hypothalamus masks. Masks of grey matter, white matter, and CSF were produced by segmenting the template EPI image in SPM. Seeds are shown in situ in Figure 8.

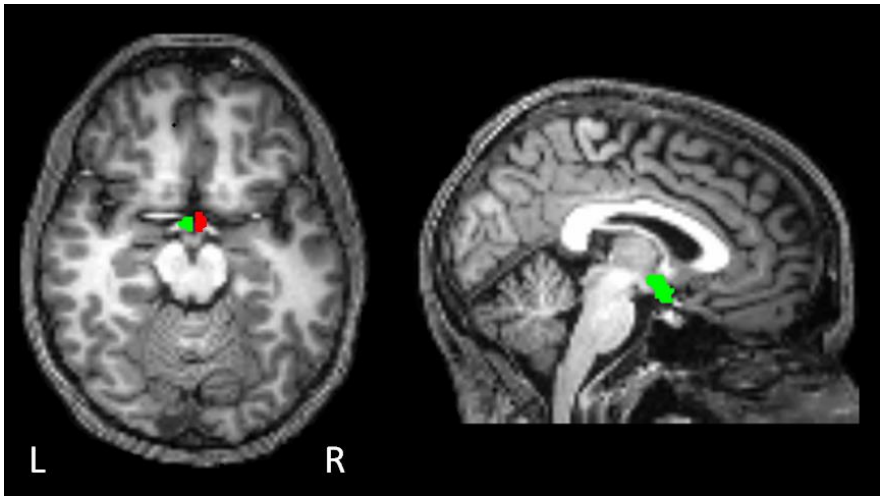

Figure 8 Left (green) and right (red) hypothalamus seeds, shown in situ on a typical participant's T1 scan.

Individual participants' realignment parameter files were added as first level covariates. Data were bandpass filtered from 0.008-0.09 Hz to remove noise and low frequency drift. White matter and CSF signals were entered as confounds, and automatically eliminated using linear regression.

First-level analysis utilised a paired t-test design with session (fasted and fed) as the independent variable. One-sided t-tests were employed to examine changes in seed-to-voxel connectivity between sessions at the second-level. A $p < .05$ FWE-corrected threshold was applied to the results in order to account for multiple comparisons. Δ glucose (the difference in readings between the sessions) was added as a second level covariate.

4.3.3 Statistical analysis

SPSS v.22 (IBM Corp., NY, USA) was used for statistical analysis. The BMI data failed the Kolmogorov-Smirnov normality test, so the nonparametric Spearman's Rho was used for the BMI correlations. Other correlational data are derived from Pearson's analyses. All reported paired t-test and correlation p-values have been adjusted for multiple comparisons using the Holm-Bonferroni correction (Gaetano 2013; Holm 1979), a sequentially rejective technique which controls for the increased risk of a type I error without unnecessarily inflating the risk of a type II error (Bender and Lange 2001; Gordi and Khamis 2004; Wright 1992).

All regression variables were standardised prior to analysis by subtracting the mean and dividing by the standard deviation (Milligan and Cooper 1988; Mitrushina et al. 2002) due to large differences in variance between the variables. This resolved the unequal variance issue (Levene's test $p = .88$).

4.4 Results

4.4.1 Glycaemia and behavioural results

Paired t-tests were employed to analyse all glycaemia and behavioural results. Before the fasted scan, participants recorded significantly lower blood glucose levels (mean fasted reading = 5.09 mmol/L ± 0.35 mmol/L; mean fed reading = 6.8 ± 0.72 mmol/L; $t(17) = -10.03$, $p < .001$), and reported feeling significantly more hungry (mean increase of 53.2 ± 20.5 on the VAS; $t(17) = 12.53$, $p < .001$). They were also significantly more distracted by thoughts of food or eating during the fasted scan (mean fasted score = 2.2; mean fed score = 1.2; $t(17) = 4.21$ $p < .001$). The POMS scores were not significantly different between sessions ($t(17) = -.372$, $p > .05$).

4.4.2 Functional connectivity results

When participants were fasted (contrast 'fasted > fed'), we observed enhanced functional connectivity between left hypothalamus and right inferior frontal gyrus (IFG). When fed, (contrast 'fed > fasted'), there was enhanced functional connectivity between right hypothalamus and a cluster in left superior parietal cortex (SPC), spanning left postcentral gyrus and precuneus. The MNI co-ordinates and t-scores are shown in Table 4; seeds and functional connectivity maps are presented in Figure 9. The addition of Δ blood glucose as a second level covariate eliminated the results clusters for both contrasts, but this appears to be just a consequence of the fasted and satiated conditions; there were no significant correlations between either change in connectivity and glycaemia ($p > .05$).

Fasted > Fed

Seed	Cluster	Cluster MNI x, y, z (mm)	K	T (1, 18)	P-FWE cluster
L Hypothalamus	R IFG	36, 32, -14	127	5.56	0.042

Fed > Fasted

Seed	Cluster	Cluster MNI x, y, z (mm)	K	T (1,18)	P-FWE cluster
R Hypothalamus	L SPC	-6, -58, 68	162	2.33	0.011

Table 4 K = cluster size (voxels). L = left. R = right. MNI = Montreal Neurological Institute. FWE = Family-wise error. MNI co-ordinates refer to the peak activated voxels.

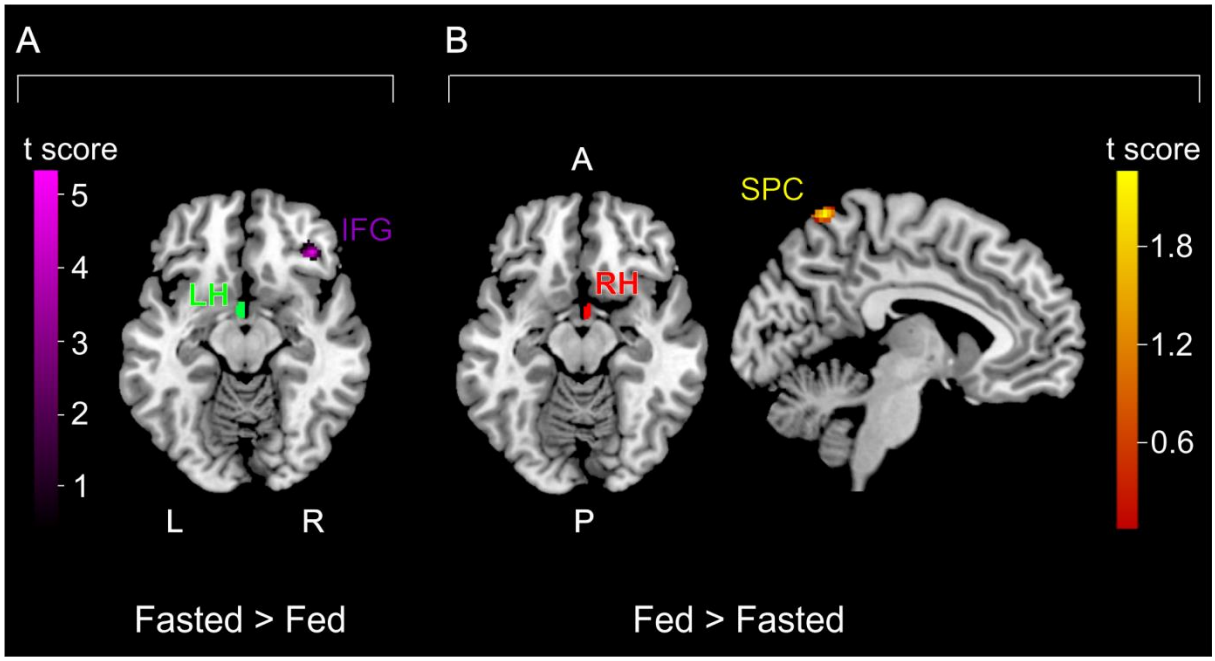

Figure 9 Panel A shows the left hypothalamus seed (LH; green) and IFG functional connectivity result (purple) on a single horizontal slice. LH and IFG are more strongly connected during the fasted session (contrast fasted > fed). Panel B shows the right hypothalamus mask (RH; red) on a horizontal slice, and the SPC functional connectivity result (yellow) on a sagittal slice. RH and SFG are more strongly connected during the fed session (contrast fed > fasted). L = left. R = right. A = anterior. P = posterior.

4.4.3 Correlation results

During the fed session, the correlation between right hypothalamus / SPC functional connectivity and the Cognitive Restraint scale of the TFEQR18 was significant ($r = -.662$, $p < .01$). During the fasted session, the correlation between left hypothalamus / IFG functional connectivity and the Cognitive Restraint scale of the TFEQR18 was also significant ($r = -.514$, $p < .05$). Additionally, there was a significant negative correlation between left hypothalamus / IFG functional connectivity and BMI ($r_s = -.529$, $p < .05$). Correlations are presented in Figure 10.

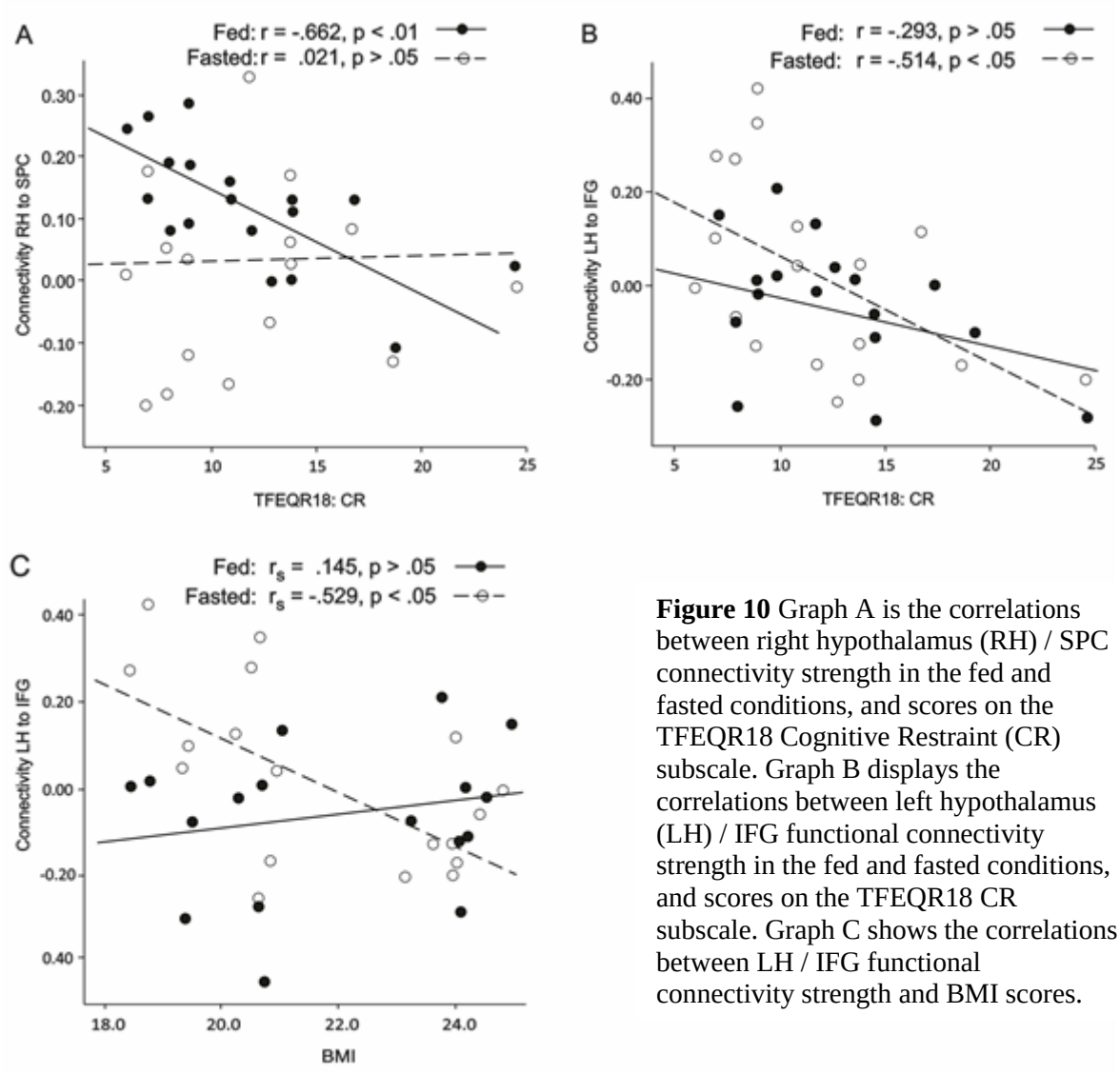

Figure 10 Graph A is the correlations between right hypothalamus (RH) / SPC connectivity strength in the fed and fasted conditions, and scores on the TFEQR18 Cognitive Restraint (CR) subscale. Graph B displays the correlations between left hypothalamus (LH) / IFG functional connectivity strength in the fed and fasted conditions, and scores on the TFEQR18 CR subscale. Graph C shows the correlations between LH / IFG functional connectivity strength and BMI scores.

An additional multiple regression using the Enter method was conducted to see if BMI could be predicted from the fasting connectivity between left hypothalamus and IFG, the fed connectivity between right hypothalamus and SPC, and the TFEQR18 Cognitive Restraint scale scores. As described in the Statistical Analysis section, each of these variables was standardised before analysis. Neither right hypothalamus / SPC functional connectivity during the fed session, nor the Cognitive Restraint subscale scores, were able to predict a significant proportion of the BMI variance ($\beta = .34$, $p > .05$; $\beta = .35$, $p > .05$, respectively). Left hypothalamus / IFG functional connectivity during the fasted session accounted for a significant amount of BMI variance ($\beta = -.50$, $p < .05$), predicting 35 % overall ($R^2 = .35$, $F(1,16) = 9.02$, $p < .01$).

4.5 Discussion

In support of our hypothesis, right and left hypothalamus resting state functional connectivities were altered by changes in homeostatic energy balance. Left hypothalamus was more strongly functionally connected to IFG during the fasted session, while right hypothalamus was more strongly functionally connected to a cluster in SPC during the fed session.

Previous studies suggest that the activation of IFG is related to cognitive control (Hare et al. 2009; Sundermann and Pfleiderer 2012). It is involved in suppressing the desire for food (Hollmann et al. 2012) and successfully resisting temptation (Lopez et al. 2014), and is more strongly activated by food stimuli in successful weight loss maintainers than in obese or normal weight participants (Sweet et al. 2012). Additionally, IFG grey matter volume (Brooks et al. 2013) and activation to satiety (Le et al. 2009) is significantly reduced in obesity. In the current study, IFG was more strongly functionally connected to left hypothalamus when participants were fasted. This enhanced functional connectivity was significantly correlated with Cognitive Restraint subscale scores on the TFEQR18, and those with a higher BMI showed less fasting functional connectivity between left hypothalamus and IFG. A relationship between hypoactivation of prefrontal cortices and elevated BMI has previously been observed (Le et al. 2006; 2007; Page et al. 2011; Volkow et al. 2009; Willeumier et al. 2011), and our results show that a significant amount of BMI variance can be explained

by the fasting functional connectivity between left hypothalamus and IFG, at least in our population of healthy participants. Rather than driving food consumption when there is an energy deficit, IFG appears to attempt to ensure that overfeeding does not occur. It seems likely that the hypothalamic drive to eat is being tempered (Tataranni et al. 1999), though the analysis method we used does not allow for the specification of directional modulation.

When participants were fed, right hypothalamus was more strongly functionally connected to a cluster in SPC which encompassed postcentral gyrus and precuneus. Following a fast, precuneus is activated by sips of a palatable liquid (Spetter et al. 2014), but deactivated in response to satiation (Gautier et al. 2000). Consciously suppressing food craving also reduces activation in precuneus (Yokum and Stice 2013). Both areas are altered in obesity; left postcentral gyrus grey matter volume is reduced (Brooks et al. 2013), and precuneus shows a reduced response to visual food stimuli (Heni et al. 2014).

Taking this and previous findings into account, the enhanced functional connectivity that we observed appears to have been modulated by the homeostatic energy balance. In the absence of an energy deficit, it is possible that the functional connectivity between right hypothalamus and SPC might represent the suppression of the eating drive. The strong correlation between this functional connectivity during the fed session and the Cognitive Restraint subscale of the TFEQR18 lends support to this hypothesis. It might also be the case that when the energy balance was restored, the participants were able to move their attention away from food. Participants reported being significantly less distracted by thoughts of food or eating during the fed session, and SPC is considered to be a core area involved in many types of attentional processing (Corbetta et al. 2000).

The current research was designed as an exploratory study, and as such we only tested participants with a BMI within the normal range. The paradigm needs to be extended to involve participants with a much wider range of BMIs in order to shed further light upon the function of this homeostatic energy balance mechanism and its relationship to the risk of being overweight. Future research involving the selective stimulation of IFG when fasting and / or SPC when feeding could further address the

role of these structures in eating behaviour. Short-term transcranial direct current stimulation of dorsolateral prefrontal cortex in normal weight males has already reported some success in calorie intake reduction (Jauch-Chara et al. 2014).

4.5.1 Conclusion

These enhanced functional connectivities seem to form part of a homeostatic energy balance network that is related to cognitive restraint of eating; preventing overeating when energy is depleted, and ending feeding or transferring attention away from food upon satiation. In our healthy weight cohort, BMI appears to be partly dependent on hypothalamic functional connectivity during acute homeostatic energy depletion. Whether this relationship holds true in overweight or obese people remains to be determined.

Chapter 5

Heightened eating drive and food stimuli attenuate central nociceptive processing

This experiment investigated the cortical structures underlying the competition between hunger / food stimuli and pain, using EEG source analysis of laser-evoked potentials.

It was published in the Journal of Neurophysiology (2015), vol. 113, pages 1323-1335. The format, but not the content, has been altered to match the style of the thesis.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak. Xiaoyun Li and Nicholas Fallon assisted with the data collection. Andrej Stancak provided training on EEG recording and data analysis. I analysed the data, interpreted the results, and wrote the manuscript. Xiaoyun Li, Nicholas Fallon, Timo Giesbrecht, Anna Thomas, Joanne Harrold, Jason Halford, and Andrej Stancak contributed useful comments on the manuscript.

Acknowledgements

The authors gratefully acknowledge Dr. Caroline Steele for providing the images used in this study, and Mrs. Nicola Williams for her technical assistance.

5.1 Abstract

Hunger and pain are basic drives that compete for a behavioural response when experienced together. To investigate the cortical processes underlying hunger-pain interactions, we manipulated participants' hunger and presented photographs of appetising food or inedible objects in combination with painful laser stimuli. Fourteen healthy participants completed two EEG sessions: one after an overnight fast, the other following a large breakfast. Spatio-temporal patterns of cortical activation underlying the hunger-pain competition were explored using 128-channel EEG recordings, and source dipole analysis of laser evoked potentials (LEPs). We found that initial pain ratings were temporarily reduced when participants were hungry compared to when fed. Source activity in parahippocampal gyrus was weaker when participants were hungry, and activations of operculo-insular cortex, anterior cingulate cortex, parahippocampal gyrus, and cerebellum were smaller in the context of appetitive food photographs than in that of inedible object photographs. Cortical processing of noxious stimuli in pain-related brain structures is reduced and pain temporarily attenuated when people are hungry or passively viewing food photographs, suggesting a possible interaction between the opposing motivational forces of the eating drive and pain.

5.2 Introduction

Hunger and pain have basic homeostatic components, and both necessitate alleviative actions. When experienced singularly the course of action is obvious, but when experienced together, the appropriate response may be less apparent. If the hunger drive is present but weak, it should be possible to ignore it and escape from the pain. If mild pain is present but the hunger drive is stronger, it should be possible to eat while ignoring pain. If either drive is sufficiently pressing, the other may not even receive conscious consideration.

Abundant evidence shows that eating (Aloisi and Carli 1996; Casey and Morrow 1983; Foo and Mason 2005; Kakeda et al. 2010; LaGraize et al. 2004; Schobel et al. 2012; Segato et al. 1997; Zmarzty et al. 1997), or expecting to eat (Dum and Herz 1984), can reduce pain. This effect is present in even very young infants, both animal (Blass et al. 1987; Blass and Fitzgerald 1988; Blass et al. 1991; Blass and Shide 1994; Ren et al. 1997; Shide and Blass 1989), and human (Blass and Hoffmeyer 1991; Blass and Shah 1995; Bucher et al. 1995; Lehr et al. 2014; Shah et al. 2012; Yilmaz et al. 2014). The anti-nociceptive effect of feeding may be at least partly attributable to activation of vagal nerve afferents as the stomach stretches; this inhibits perception of several types of pain (Faris et al. 2006; Sedan et al. 2005). Conversely, a smaller body of evidence shows that fasting can produce analgesia (Davidson et al. 1992; de los Santos-Arteaga et al. 2003; McGivern et al. 1979; McGivern and Berntson 1980). Interestingly, fasting is employed as a treatment for chronic pain in some settings (Michalsen and Li 2013; Michalsen et al. 2002, 2005; Wilhelmi de Toledo et al. 2013).

One factor playing a role in fasting or feeding-related analgesia might be the endogenous opioid system, as studies which administered naltrexone or naloxone found that the analgesia effect was reversed (Blass et al. 1987, 1991; Blass and Fitzgerald 1988; Davidson et al. 1992; de los Santos-Arteaga et al. 2003; Dum and Herz 1984; McGivern et al. 1979; McGivern and Berntson 1980; Segato et al. 1997; Shide and Blass 1989). Such compounds also reduce the pleasure normally derived from ingesting palatable foods (Drewnowski et al. 1995; Fantino et al. 1986; Halford et al. 2010; Schneider et al. 2010). Endogenous opioids are intricately involved in feeding regulation (see Bodnar 2004 for a comprehensive review), and levels of β endorphins

rise when eating highly palatable food (Dum et al. 1983; Mercer and Holder 1997). Fasting induces a decrease in endogenous opioid levels but an increase in beta-hydroxybutyrate (Pan et al. 2000), an isomer of the illicit drug gamma-hydroxybutyrate (Brown 2007). Gamma-hydroxybutyrate produces a considerable rise in dopamine release (Gessa et al. 1966; Itzhak and Ali 2002). Dopamine appears to play a significant role in analgesia (Jarcho et al. 2012; Wood 2006) and reductions in affective responses to pain (Jarcho et al. 2012; Tiemann et al. 2014), and enhanced dopamine levels are diminished by naloxone (Klitenick et al. 1992; Seilicovich et al. 1985; Snead and Bearden 1980). The evidence reviewed above suggests that both physiological and hormonal factors play a part in food-related analgesia.

If hunger can override pain and vice versa, a mechanism can be postulated that allows attending to one of these drives at the expense of the other. Pain, feeding, and taste pathways are represented in the brainstem (Craig 2003b; Norgren 1990; Small et al. 2003), hypothalamus (Burton et al. 1976; Dafny et al. 1996; Guyton and Hall 2004), and amygdala (Bernard and Besson 1990; Bernard et al. 1992; Neugebauer and Li 2002; Sanghera et al. 1979). Additionally, the pain and taste pathways are represented in S1 (Gingold et al. 1991; Kenshalo et al. 1980; Norgren 1990), anterior insula (Dostrovsky and Craig 1996; Pritchard et al. 1986; Scott et al. 1986; Sudakov et al. 1971), and ACC (Bushnell and Duncan 1989; Hutchison et al. 1999; Sikes and Vogt 1992). Any of these areas could therefore facilitate a competitive interaction between hunger and pain.

Rewarding stimuli other than food, such as monetary gain (Becker et al. 2013), and the expectation of analgesia (Elsenbruch et al. 2012), have also been shown to inhibit the perception of pain. According to the motivation-action theory of emotion (Bradley et al. 2001; Lang et al. 1992, 1997), two motivational systems are posited to exist: one appetitive, the other defensive. The appetitive system is engaged by approach-related reinforcers such as ingestion; the defensive system is triggered by threat.

The aim of this study was to explore competitive interactions between the appetitive and defensive motivational systems, using fasted vs. fed states, and presenting food photographs in combination with painful laser stimuli. We hypothesise that viewing food cues should activate the appetitive system, inhibiting the pain-

activated defensive system and resulting in a suppression of subjective pain perception and pain-related brain activity. Provided that the visual food cues are sufficiently motivationally salient, such a pain decrease is also predicted by the motivation-decision model of pain modulation (Fields 2007), whereby endogenous opioids suppress responses to noxious stimuli to allow responding to more important motivational stimuli. Such a reciprocal relationship between pain and other motivational stimuli, such as fear and social cues, has already been described (Wiech and Tracey 2013), but this is the first study to investigate brain areas involved in facilitating competition between the motivational drives of appetite and pain, using source reconstruction of LEPs.

5.3 Methods

5.3.1 Participants

Fourteen healthy volunteers (seven male) with a normal BMI (World Health Organization 2006) from the undergraduate and postgraduate student population of the University of Liverpool took part in this study. The average age of the participants was 24.6 ± 4.1 (mean ± SD). Participants gave their written informed consent and the study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the University of Liverpool Research Ethics Committee.

5.3.2 Procedure

Participants were asked on the day before both sessions not to exercise more than they would normally, and not to eat or drink anything other than water from midnight. Compliance was assessed using diary entries; no participants reported taking part in any strenuous exercise or excessive eating. Participants completed two sessions, which were separated by an average of 7.5 days (± 5.6), and attended the lab at 8.45 am after a 9 hour overnight fast. Participants either remained fasted for the remainder of the session (fasted condition), or were provided with a standardised breakfast (fed condition). Session order was counterbalanced across participants. The breakfast consisted of cornflakes, semi skimmed milk, toast, margarine, jam, orange juice, and tea / coffee.

The total energy content was 531 kcal (2223 kJ) for females, and 680 kcal (2847 kJ) for males. Measures of hunger, desire to eat, and prospective consumption were taken using 100 mm VAS immediately prior to the EEG recording in both the fasted and fed sessions.

The experiment took place in a sound attenuated room. Painful stimuli were produced using an Nd-YAP laser stimulator (Stim1340, El.En., Italy); the spot size was 5 mm, and the pulse duration was 3 ms. Before beginning the experiment a 5 cm circle was drawn on the dorsal surface of the participants' right hand, and the laser stimuli were applied pseudo randomly within. The laser intensity to be used was selected by applying a succession of painful stimuli ranging from 1.0 J up to 2.25 J. Intensities started off low, and after each stimulus the participant was asked to rate, on a scale of 1 to 7, how much pain they had felt. The scale was anchored as '1: no pain' and '7: worst imaginable pain'. The laser intensity was gradually increased until participants gave a rating of 3-4 (moderate pain). When this rating was achieved, three more stimuli at this intensity were applied to ensure that the rating remained consistent. Participants were given five practice trials and shown how to make their response.

The experiment was comprised of three blocks, each containing 32 trials. Figure 11 shows a visual representation of the trial structure. We divided the experiment into blocks for reasons of safety and data quality. As upwards of 90 laser stimuli were applied to the dorsum of the hand, it was prudent to check for skin burns or abnormal skin reactions between successive blocks of stimuli. The stimulation period took about 40 minutes and therefore, to avoid fatigue and any discomfort due to limited mobility, resting periods also served to allow participants to stretch and relax. Finally, the electrode sponges required regular remoistening in order to keep electrical impedance low. The approximate amount of time between blocks ranged from four to eight minutes, depending on the time it took to re-saturate the electrodes and / or adjust the laser intensity if participants' pain ratings had noticeably decreased.

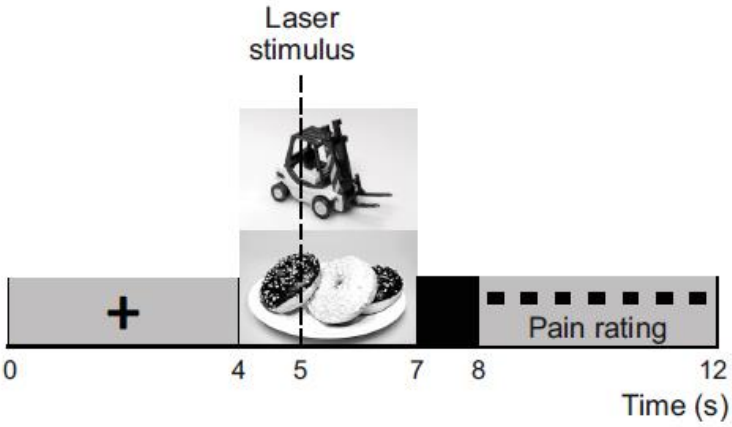

Figure 11. Trial structure. Each trial began with a fixation cross 4000 ms long, which was replaced by a photograph of either food or an inedible object. The photograph was visible for 3000 ms, and a laser stimulus was applied exactly 1000 ms after the onset of the photograph. The screen went blank for 1000 ms, and then participants rated the pain they had felt on a visual analogue scale of 1-7 (anchored as 1 = no pain; 7 = worst imaginable pain). The scale was incremented by repeatedly clicking the mouse. Equal numbers of food and object photographs were presented within each block.

The food photographs used in the experiment were selected on the basis of an unpublished pilot study, which had found them to be consistently rated as tasty, enjoyable, and filling. Object photographs (included as the control condition) were a mixture of household and leisure items, unrelated to food or eating. Photographs were 492 x 329 pixels, with a resolution of 72 dpi. Participants were seated 60 cm away from the 19 inch LCD monitor. Delphi 7 (Borland Software, Austin, TX) was utilised as the stimulus presentation program.

At the end of each experimental session, participants rated each food in the photographs for how tasty, filling, and enjoyable it looked, along with how much they would eat of it if they were hungry. Ratings were made on numeric rating scales from 0 to 10.

5.3.3 Recordings and data analysis

EEG was recorded continuously using the 128-channel Geodesics EGI System (Electrical Geodesics, Inc., Eugene, OR, USA). The anatomical landmarks of the nasion, inion, and left and right preauricular points were used to position the sensor net. Electrode to skin impedances were kept below or equal to 50 kΩ, and checked between each block. If necessary, the electrode sponges were remoistened. The bandpass filter

was 0.1 to 200 Hz and the sampling rate was 1000 Hz. Electrode Cz was used as the reference. BESA 6.0 (MEGIS GmbH, Germany) was used for data processing. Raw data were transformed into reference-free data using common average reference (Lehmann 1987). The data were filtered from 0.5 to 70 Hz, with an additional notch filter applied at 50 Hz. Oculographic and electrocardiographic artifacts were removed using principal components analysis (Berg and Scherg 1994), and the data were visually inspected to check for other artifacts. Trials contaminated by additional artifacts were excluded from the analysis.

To analyse the typical period of nociceptive processing the data were divided into epochs from -100 to +600 ms relative to the onset of the laser stimulus, with -100 to 0 ms used as the baseline. LEPs were computed separately for food and object photographs by averaging relevant trials firstly across single blocks, then averaging the block averages across single sessions. This yielded, for each participant, four overall LEPs: one when hungry and viewing food photographs, one when hungry and viewing object photographs, one when fed and viewing food photographs, and one when fed and viewing object photographs. The average number of trials accepted per participant for these four conditions were (respectively, out of a possible 48): 38.3 ± 8.1, 37.4 ± 8.8, 38.5 ± 6.1, and 37.3 ± 6. Finally, the individual participant averages were combined to produce group-level condition averages. Additionally, in order to explore the group-level effects of the appetite manipulation independently of the photograph type, all subjects' hungry condition trials were collapsed across photograph types to produce averaged LEPs for the hungry condition, and all subjects' fed condition trials were collapsed across photograph types to produce averaged LEPs for the fed condition.

5.3.4 Source dipole reconstruction

After calculating the subject and group level averaged potentials, sources were estimated from the group-level averaged waveforms using classical LORETA analysis recursively applied (CLARA: Hoechstetter et al. 2010), which takes as a first step a regularised LORETA (Low Resolution Electromagnetic Tomography: Pascual-Marqui et al. 1994) image, then in iterative steps, smooths the previous image and sets to zero all voxels with amplitudes of less than 10% of the maximum activation, effectively

eliminating them from the analysis. This produces an image containing an amplitude of activation defined for each voxel. A LORETA image taking this voxel-specific information into account is then computed.

Parameters specified in the current CLARA analysis were singular value decomposition (SVD) regularisation with a cut-off of 0.01%, two iterations, and a voxel size of 7×7×7mm[5.] A significant advantage of using CLARA is that the elimination of voxels with small amplitudes gives rise to a far clearer and more circumscribed image than some other source analysis techniques, making it less problematic to visualise sources that are spatially close. The spatial maxima of activation clusters produced by CLARA were used as seeds for fitting a set of equivalent current dipoles. The orientations of equivalent current dipoles were fitted separately both in grand average and individual LEPs.

The 4-shell ellipsoid head model was employed, using the following conductivities (S/m = Siemens per meter): brain = 0.33 S/m; scalp = 0.33 S/m; bone = 0.0042 S/m; cerebrospinal fluid (CSF) = 1.0 S/m.

5.3.5 Statistical analysis

Unless otherwise stated, SPSS v.20 (IBM Corp., NY, USA) was utilised to perform statistical analyses. Repeated measures ANOVAs were used to test for the effects of sessions, photographs, and blocks on the source dipole waveforms and pain ratings. Paired Student's t-tests were used to compare sessions and photographs. All p values were Bonferroni corrected in order to account for multiple comparisons, and a 95% confidence level was employed throughout. Pearson's correlation coefficients were computed to test for correlations between source dipole moments and various self-report measures. Differences between correlation coefficients were evaluated using Statistica 7.0 (StatSoft, Inc., Tulsa, USA).

Due to the absence of previous studies addressing the effects of appetite on LEPs, we did not identify any specific LEP component or latency period of interest *a priori*. Instead we employed a data-driven analysis, which allowed the detection of clusters of signals and intervals of interest manifesting the effects of one or more

independent variables and their interaction. To this end the source dipole waveforms were analysed using repeated measures ANOVA and a random permutation test (Maris and Oostenveld 2007) in Matlab v. 7.8 (MathWorks Inc., Natick, MA, USA), using 1000 permutations and a 99% confidence level. The permutation method was necessary to control for the inflated risk of Type I error, brought about by the large number of ANOVA tests required.

5.4 Results

5.4.1 Behavioural data

The goal of giving participants a large breakfast was to ensure that they felt full and not hungry, leading to clearly delineated hungry and fed conditions. Paired t-tests were employed to compare the level of eating drives between sessions. When participants were fed in comparison to fasting, VAS ratings were significantly lower for hunger (mean ± SD for hungry session: 62.79 ± 15.5; for fed session: 9.43 ± 15.8, $t(13) = 11.1$, $p < .001$), desire to eat (mean ± SD for hungry session: 64.93 ± 19.6; for fed session: 8.79 ± 11.8, $t(13) = 9.9$, $p < .001$), and prospective consumption (mean ± SD for hungry session: 57.79 ± 18.3; for fed session: 15.86 ± 16.3, $t(13) = 8.0$, $p < .001$).

Laser intensities were necessarily adjusted between blocks for most of the participants, due to them becoming habituated to the laser and reducing their subjective responses to the noxious stimuli. Collapsing the laser intensities across blocks revealed that the laser intensity was almost identical in both conditions; the mean intensity used was 2.45 J ± 0.35 J in the hungry condition and 2.47 ± 0.31 J in the fed condition. This produced a mean intensity difference of 0.02 J between conditions, which was statistically not significant ($t(13) = -1.1$, $p = .298$).

Pain ratings were analysed using a 2 (sessions) × 2 (photographs) × 3 (blocks) ANOVA for repeated measures. Mean pain ratings did not significantly differ between the sessions (hungry and fed, collapsed across all photographs and all blocks). Nor were they statistically different across the two photograph types (collapsed across all blocks). There was, however, a statistically significant effect of blocks on mean pain ratings ($F(2,26) = 4.49$, $p < 0.05$); and a statistically significant interaction between session and

blocks (F (2,26) = 5.72, p < 0.05). As shown in Figure 12, an analysis of simple effects revealed that pain ratings in the fed session in block 1 were significantly larger than those in blocks 2 (t (13) = 5.4, p < 0.05) and 3 (t (13) = 5.1, p < 0.05). Blocks 2 and 3 were not significantly different from each other. There was a statistically significant difference between the pain ratings during block 1 across sessions (t (13) = 2.16, p < 0.05).

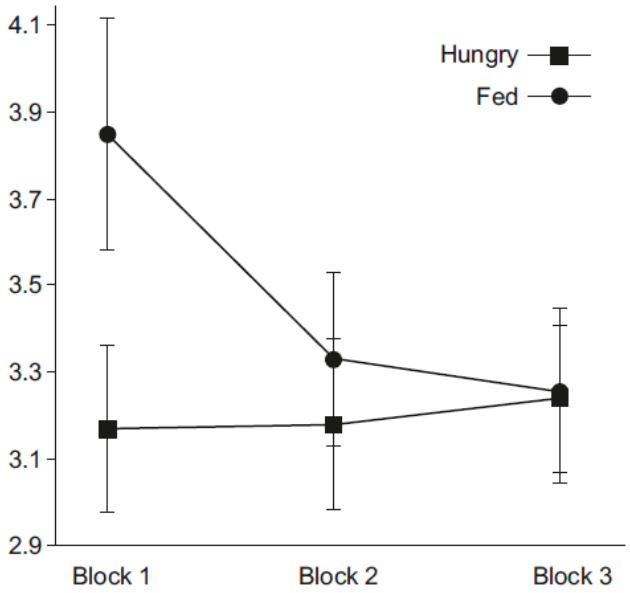

Figure 12. Mean pain intensity ratings ± standard deviation bars during the hungry and fed sessions, averaged by block. There is a significant difference in block 1 between the mean pain ratings in the hungry session (3.17 ± 0.74) compared to the fed session (3.85 ± 1.0).

Student's paired t tests were used to compare the food photograph ratings obtained after both EEG recordings. There were no statistically significant differences between the sessions (p > .05).

5.4.2 Source dipole model of LEPs

Figure 13A shows the grand averaged waveforms and topographic maps of LEPs at different equivalent current dipoles localised using CLARA, on data combined from all sessions, photographs, and blocks. Figure 13B illustrates the spatial clusters obtained in CLARA analysis, and locations of equivalent current dipoles which were fitted based on CLARA. The source model of the LEPs accounted for 94.34% of the variance. It was

best constructed by six source dipoles; adding another source did not explain significantly more of the variance. Table 5 A-C shows anatomical labels and approximate Talairach coordinates of each of the six source dipoles.

A

Anatomical label	**Talairach (mm)**	**Epoch (ms)**	**Hungry mean ± SD**	**Fed mean ± SD**	**F**	**p**
Right PHG (3)	27, -9, -16	248 - 257	16.32 ± 25.0	27.51 ± 21.9	5.10	.042

B

Anatomical label	**Talairach (mm)**	**Epoch (ms)**	**Food mean ± SD**	**Object mean ± SD**	**F**	**p**
Left OIC (1)	-37, -12, -2	150 – 160	19.30 ± 19.3	21.92 ± 18.2	7.18	.019
ACC (2)	-3, -21, 45	167 – 177	9.79 ± 15.0	15.00 ± 15.2	7.06	.020
Right PHG (3)	27, -9, -16	300 – 330	27.75 ± 15.9	35.00 ± 14.5	16.08	.001
Cerebellum (6)	18, -50, -35	395 – 405	14.00 ± 10.8	19.61 ± 15.3	5.18	.040

C

Anatomical label	**Talairach (mm)**	**Epoch (ms)**	**Hungry / Food**	**Fed / Object**	**F**	**P**
Left PHG (4)	-27, -9, -21	-	-	-	-	-
MFG (5)	-3, 49, 8	-	-	-	-	-

Table 5. The anatomical labels of the sources (source numbers shown in brackets), with corresponding approximate Talairach co-ordinates in mm (x, y, z) and time epochs of significant modulations. Mean values refer to the mean amplitude of the source waveform during the time interval when it was significantly modulated by a session or photograph factor. Section A shows the source that was significantly modulated by session; section B shows sources that were significantly modulated by photograph type. 'Food' and 'Object' refer to the food and object photograph types. Section C shows sources that were not significantly modulated during any time epochs by either experimental manipulation, but still contributed significantly to the source dipole model. OIC = operculo-insular cortex.

Source 1 was tangentially oriented and located in left operculo-insula cortex. Its shortest latency peak occurred at 154 ms, with a negative maximum over the temporal electrodes. Both the topographic pattern and the peak latency suggest that this component is equivalent to the N1 component of LEPs. Source 1 peaked again at 188 ms (topographic map not shown) during the period in which the N2 component is known to operate. Source 2 was associated with a strong negative scalp potential field at the vertex peaking at 181 ms, corresponding to the N2 component of LEPs. It was a radially oriented dipole located in the anterior mid-cingulate cortex. Sources 3 and 4 were located in bilateral parahippocampal gyri (PHG). Source 3 showed two peaks, one at 281 (topographic map not shown) and one at 327 ms; source 4 peaked once at 272 ms. They were obliquely oriented, and pointed posteriorly towards the positivity at posterior parietal electrodes. Source 5 was fitted in the right middle frontal gyrus (MFG), and accounted for a negativity seen in the right frontal electrodes at 248 ms. Sources 3, 4, and 5 contributed to the large P2 LEP component. Finally, CLARA also identified the presence of a comparatively weak source in the right cerebellum, peaking at 404 ms. Source 6 had a tangential orientation and explained part of the negative spatial maxima seen in electrodes placed on the lower face. The peak latency of source 6 would correspond to the P2 or N3 component of LEPs (Stancak et al. 2013). Although one previous paper from our laboratory has indicated the presence of a cerebellar component in LEP data (Stancak and Fallon 2013), this source dipole will need further independent confirmation, and therefore any conclusions based on modulations of source 6 should be treated with caution.

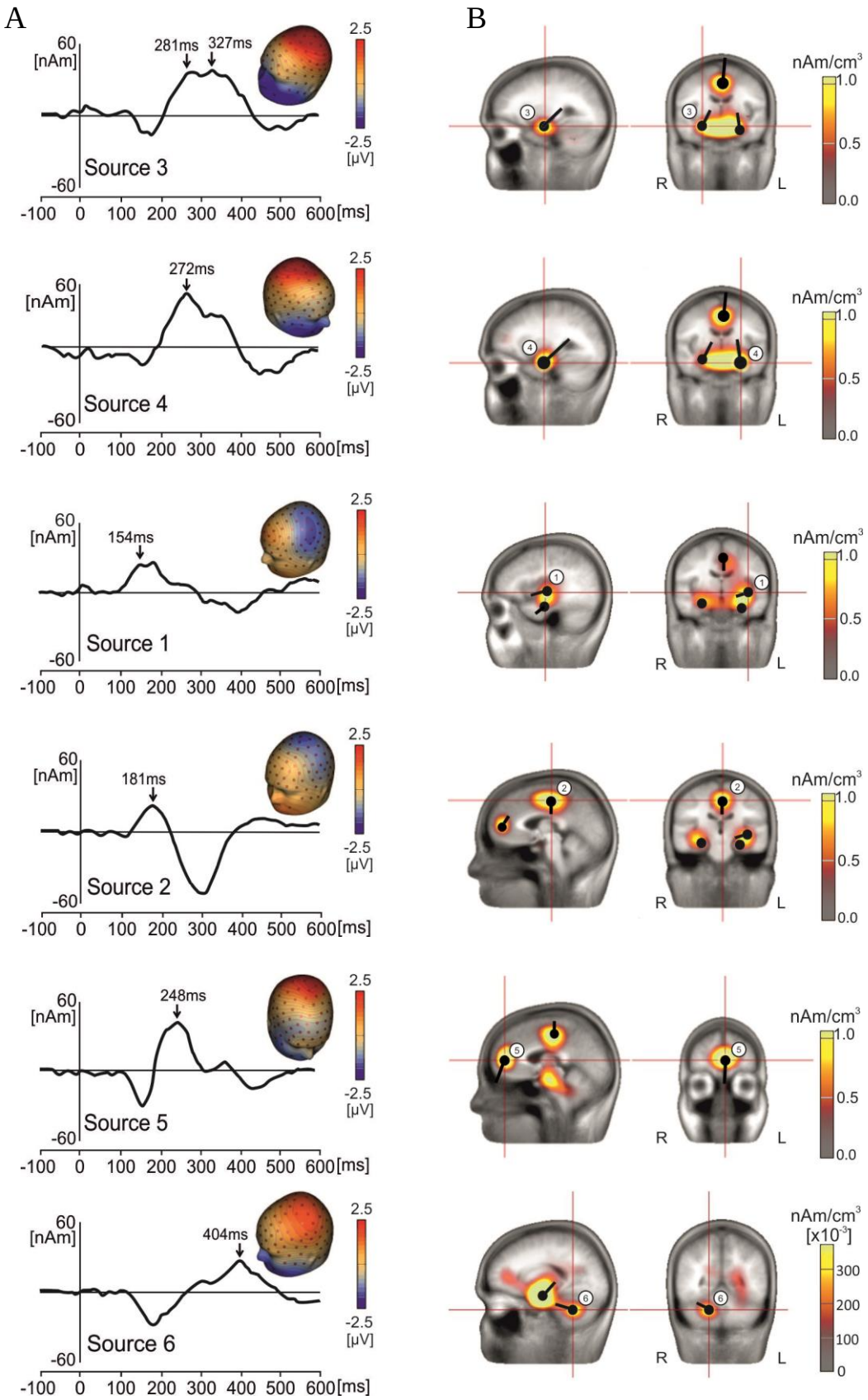
A
B
281ms
327ms
Source 3
272ms
Source 4
154ms
Source 1
181ms
Source 2
248ms
Source 5
404ms
Source 6
60
[nAm]
-60
-100 0 100 200 300 400 500 600[ms]
2.5
-2.5
[μV]
nAm/cm3
1.0
0.5
0.0
R
L
[x10-3]
300
200
100
0

Figure 13. Panel A: waveforms of sources 1 to 6, collapsed across both sessions (hungry and fed) and both photograph types. Peak latencies are denoted by an arrow. Panel B: sources 1 to 6 superimposed over a standard structural MRI scan. In each image, the source corresponding to the waveform in panel A is indicated by its number, and by the red crosshairs.

5.4.3 Effects of sessions and photographs

To test the effects of sessions and photographs on LEPs, a two-way ANOVA for repeated measures was carried out for all six waveforms over the entire time interval from -100 to 600 ms. To reduce the chances of a Type I error, a permutation analysis (Maris and Oostenveld 2007) was carried out. Figure 14A shows the only statistically significant effect of session, which was seen in source 3, located in the right PHG. In the time interval of 248–257 ms, the source activity was stronger in the fed session than in the hungry session. Mean values of source 3 amplitudes in the hungry and fed sessions, as well as F and p values, are given in Table 5A. Figure 14B shows the statistically significant effects of photographs on the waveforms of sources 1, 2, 3, and 6. Each of these sources showed somewhat smaller activations in the context of food photographs in comparison to object photographs. Although the statistically significant effects of photograph type were evident from 150–160 ms in source 1 and 167–177 ms in source 2, corresponding to the typical time course of the LEP N1 component, at the maximum peaks of these two sources the topographic maps were already dominated by the N2 LEP component, maximal over the vertex. Mean values of source dipole moments, time epochs of statistically significant waveform modulations, and F and p values are shown in Table 5B.

There were no effects of session or photograph type on sources 4 or 5. Their approximate Talairach co-ordinates are given in Table 5C. There were no statistically significant interactions between sessions and photographs in any of the sources ($p > .05$).

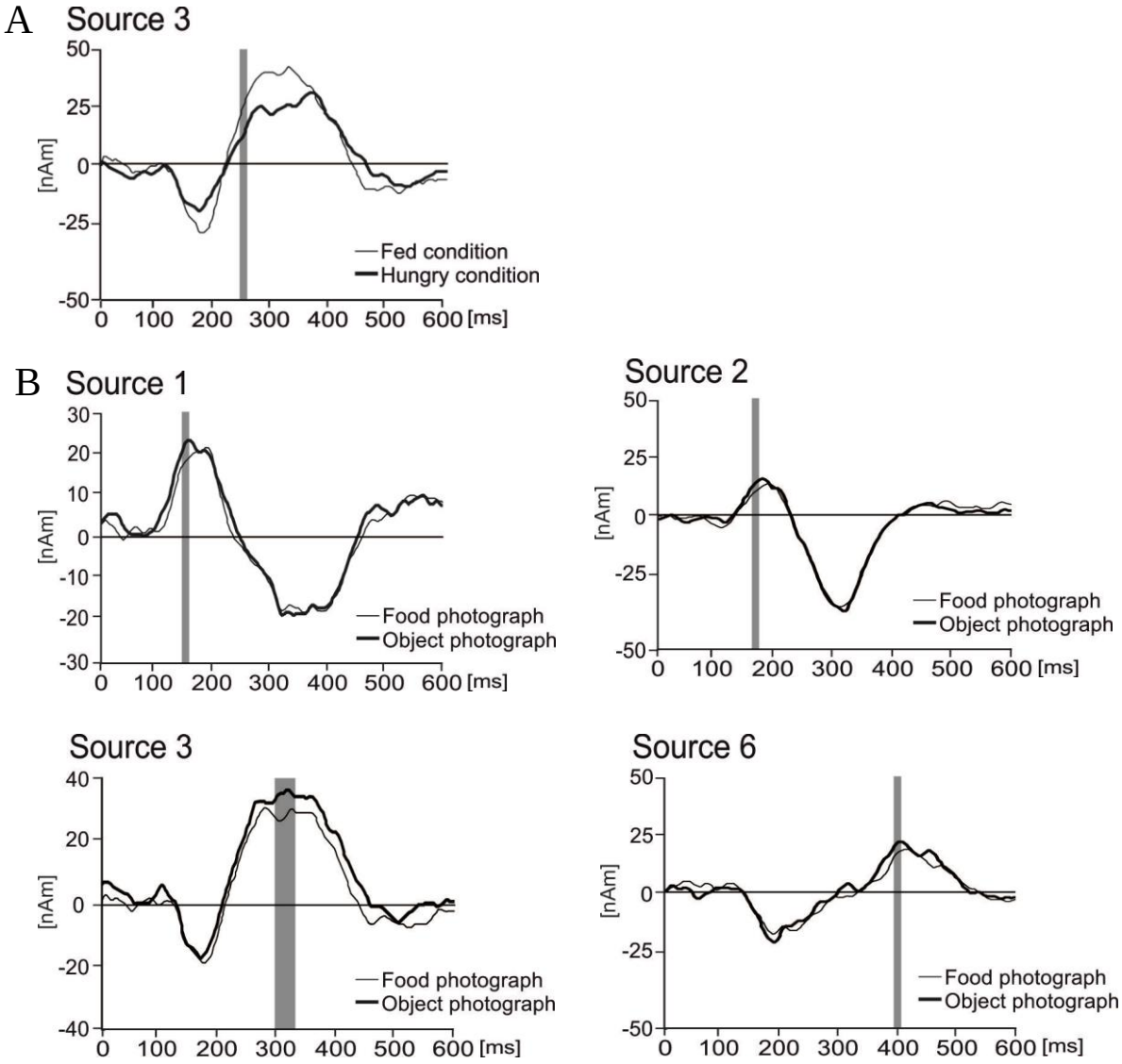

Figure 14. Source waveforms showing modulation by session (panel A), and photograph type (panel B). Time epochs of significant modulations are indicated by the grey bars. These epochs were derived from the statistical analysis of the entire LEP waveform in each condition.

5.4.4 Correlation data

After each EEG session, ratings of how enjoyable, filling, and tasty the food in the photographs looked were taken on VAS, along with a rating of how much participants could eat of the different foods if they were hungry. These measures were highly inter-correlated within sessions, so they were collapsed to give a single hedonic rating scale for each session.

To analyse the contribution of hedonicity to the activation of source 3 (right PHG), which showed a statistically significant effect of session, a Pearson's correlation coefficient was computed between hedonic ratings and the source dipole moment during the time interval 248–257 ms (averaged over blocks and photographs). The correlation was significant in the fed session ($r(11) = .625$, $p < 0.05$), and stronger during the hungry session ($r(11) = .810$, $p < 0.01$). The correlation coefficients themselves were not significantly different ($p > 0.05$). Source 3 activation during this epoch was not significantly correlated with pain ratings.

As far as pain rating is concerned, only sources 1 (operculo-insular cortex) and 6 (cerebellum) were significantly correlated with pain ratings during the time epochs of significant modulation by photograph type. Activation in the operculo-insular cortex was positively correlated with pain ratings in the context of food photographs ($r(10) = .594$, $p < 0.05$), marginally more so in the context of the object photographs ($r(10) = .603$, $p < 0.05$). Activation in cerebellum was markedly more positively correlated with pain ratings in the context of object photographs ($r(12) = .581$, $p < 0.05$) than in the context of food photographs ($r(12) = .343$, $p > 0.05$). Again, the correlation coefficients themselves were not significantly different ($p > 0.05$).

5.5 Discussion

Participants reported less pain in the initial block of the experiment and exhibited reduced source activity in right PHG when they were hungry compared to fed; similarly, viewing food photographs resulted in comparatively small but statistically significant reductions in pain-related source activations in the left opercular insula, ACC, right PHG, and cerebellum. These results are in agreement with those predicted by the theory of emotion and motivation (Bradley et al. 2001; Lang et al. 1992, 1997).

Hunger slightly attenuated pain in the first block of the experiment, and suppressed activation in the right parahippocampal region. This finding corroborates previous studies which have reported decreased nociceptive processing when fasting (Davidson et al. 1992; de los Santos-Arteaga et al. 2003; McGivern et al. 1979; McGivern and Berntson 1980). A contribution by parahippocampal cortex (or very

closely related areas) to evoked pain responses has been postulated previously in EEG source analysis studies (Stancak and Fallon 2013; Stancak et al. 2013; Valeriani et al. 1996, 2000, 2002), and experiments with fMRI have cited PHG as being involved in processes such as reactivating memories of pain (Kattoor et al. 2013), pain anticipation (Fairhurst et al. 2007), and pain sensitivity (Piche et al. 2010). Interestingly, in the current study, the effects of session on PHG activation were not correlated with pain ratings, but rather with ratings of food photograph hedonicity. Results from appetite research have found PHG to be activated by hunger (Del Parigi et al. 2002; Tataranni et al. 1999), and by appetitive stimuli when participants were fasted (Cheng et al. 2007; LaBar et al. 2001; St-Onge et al. 2005).

Passively viewing food photographs also attenuated pain responses to a small degree in operculo-insular cortex, mid ACC, PHG, and cerebellum. Insula is reliably activated in response to visually-presented appetitive stimuli (Kroemer et al. 2013; Porubská et al. 2006; Simmons et al. 2005; van der Laan et al. 2011), and also forms part of the 'pain matrix'; operculo-insular cortex responses to laser-evoked pain are almost universally reported (Garcia-Larrea et al. 2003). This region has been cited as a likely generator of N1 LEPs (Iannetti et al. 2005; Vogel et al. 2003); it is sometimes indistinguishable from secondary somatosensory cortex (SII), since the structures lie so close together and are densely reciprocally connected, and therefore both areas have been implicated as possible generators of N1 in some studies (Frot et al. 1999; Valeriani et al. 2000). The area of ACC identified in the current study is involved in orienting attention to salient stimuli (Kim et al. 2013; Peyron et al. 1999; Tölle et al. 1999), and N1 has recently been demonstrated to be augmented by salient stimuli (Ronga et al. 2013). The timing of the operculo-insular and ACC modulations (150–160 ms and 167–177 ms after the laser stimuli, respectively) by food photographs in the current study corresponds closely to the timing of the classically evoked N1 LEP (García-Larrea et al. 1997; Legrain et al. 2002; Tarkka and Treede 1993; Valeriani et al. 1996), while the peak activations at 188 and 181 ms (respectively) are approaching the usual timing of the N2 LEP component (Friederich et al. 2001; Legrain et al. 2002; Stancak and Fallon 2013). This finding corroborates previous research which has suggested that operculo-insular cortex contributes to both N1 and N2 (Garcia-Larrea et al. 2003; Tarkka and Treede 1993; Valeriani et al. 1996).

N1 has been shown to code pain intensity (Iannetti et al. 2005; Stancak et al. 2012), and, when general vigilance effects are held constant, a change in attentional focus (Legrain et al. 2002). Since participants saw the food photographs before they felt the pain, any reduced operculo-insular and ACC source activation may result from a reduced capacity of the pain to shift attention away from the food.

Right PHG activation was reduced from 300–330 ms when participants viewed food photographs (independently of session effects). This corresponds to the usual timeframe of the P2 LEP (Friederich et al. 2001; Valeriani et al. 2007; Yamasaki et al. 1999), which appears to reflect the affective processing of pain. Its amplitude can be tempered by viewing pleasing aesthetics (de Tommaso et al. 2008) and pleasant pictures (de Tommaso et al. 2009), and the magnitude of P2 (in the absence of pain) has also been demonstrated to be augmented by emotional words (Herbert et al. 2006; Schacht and Sommer 2009). The PHG has also previously been shown to respond to manipulations of emotion in the context of pain (Roy et al. 2009; Stancak et al. 2013), and more generally, as part of the entorhinal cortex, it is often described as being part of an emotion network (see Lindquist et al. 2012, for a comprehensive review). That the PHG activation corresponding to the time frame of P2 was weaker when participants were viewing food photographs suggests that appetitive stimuli might somewhat diminish the affective dimension of pain.

The final area that appeared to be modulated by photograph type was the cerebellum, between 395–405 ms. Cerebellum is cited often as a region activated by pain (Moulton et al. 2010; Peyron et al. 2000), and, in conjunction with the limbic structures ACC and PHG, is part of an emotion-related network activated in response to generally aversive stimuli (Moulton et al. 2011). Cerebellum appears to reflect both ascending and descending pain signaling (Borsook et al. 2008; Hagains et al. 2011; Saab and Willis 2003; Yelle et al. 2009); that it was temporally the last structure to participate in the source model of the LEP suggests that it was engaged here in pain inhibition. This explanation is supported by our finding of correlations between pain intensity and the strength of source activity in cerebellum.

The MFG formed a statistically important part of the source model, but its activation was not modulated by either appetite status or photograph type. The MFG is

commonly cited in pain studies (Baron et al. 1999; Brooks et al. 2002; Iadarola et al. 1998; Ochsner et al. 2006; Song et al. 2006; Tracey et al. 2000). It is significantly activated in response to pain regardless of whether the pain is strong or weak (Kong et al. 2010), and is involved in decision making (Krain et al. 2006; Rogers et al. 1999; Schmitz and Johnson 2006) and response selection (Hazeltine et al. 2003; Schumacher et al. 2003). It is therefore not surprising that the experimental manipulations failed to affect activation in MFG, since every trial in every condition required a decision and a response.

The temporal and spatial distribution of experimental effects across varying time intervals is likely due to sequential and hierarchical processing of sensory information, seen also in other sensory modalities (Hari et al. 2010). The N1 component of LEPs, originating primarily in the contralateral operculo-insular and mid ACC (Figure 14B, top panel), was consistent with an early arrival of nociceptive information via the spinothalamic tract neurons to posterior insula, secondary somatosensory cortex and the ACC, which are the targets of the spinothalamic tract neurons (Dum et al. 2009). The subsequent latency components, in particular the long-latency component peaking at 404 ms (Figure 14B, bottom panel), point to involvement of additional brain regions such as medial temporal cortex, rostral ACC and cerebellum, consistent with employment of higher order, top-down control processes at a later stage of nociceptive processing. Therefore, source dipole modelling was required to separate the spatial and temporal components of LEPs.

There was no interaction between sessions and photograph types, which suggests that there may be separate processes contributing to the comparatively small effects on pain. The first (related to session) appears to reflect generally heightened attention to any distracting stimuli when hungry, resulting in a decreased perception of pain and an increased appreciation of the hedonicity of appetitive stimuli. The second (related to photograph type) would be stimulus-driven and perhaps manifested as a more direct competition for attention between food stimuli and pain, resulting in slight interference with nociceptive processing.

Our results provide a suggestion as to a possible mechanism underlying previous findings that fasting can reduce pain in chronic pain patients (Michalsen and Li

2013; Michalsen et al. 2002, 2005; Wilhelmi de Toledo et al. 2013), though we acknowledge that the short-lasting experimental pain we employed lacks almost all of the sensory and psychological features commonly seen in chronic pain. Thus, the clinical impact of the study is limited and needs to be established in future studies involving chronic pain patients.

5.5.1 Limitations

An additional control condition of pain without the context of any kind of visual stimuli was not included in the study. The extra control would have eliminated any possibility of an effect of unequal attention between the food and object photograph conditions, potentially strengthening our results. However, a control condition not displaying complex pictures may affect nociceptive processing differently from conditions involving pictures of food or objects, due to reduced demand on attention and decreased distraction.

Secondly, pain ratings were only significantly different for the fed and fasted conditions during the first block; after this they dropped considerably (Figure 12). The decline in subjective pain ratings is most likely due to habituation. This is an often cited hazard in LEP research where many trials are required (de Tommaso et al. 2005; Mobascher et al. 2010); one study showed that the habituation effect could be mitigated by continually increasing the laser intensity (Weiss et al. 1997), but this approach was beyond the scope of this study, where it was more important to match the laser intensity across the fed and fasted sessions.

5.5.2 Conclusion

The results of the current study demonstrate that hunger and visual food stimuli may partially suppress the cortical processing of noxious stimuli, and induce a short lived and small reduction in pain intensity. The clinical significance of these findings for pain relief in chronic pain patients is limited at this stage, and will need to be established in future studies.

Chapter 6

Appetite-induced modifications of the functional connectivity of a pain network

This experiment investigated changes in the functional connectivity of areas of the pain matrix induced by manipulations of appetite.

It was written up for publication in a neuroscience journal. The format, but not the content, has been altered to match the style of the thesis.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak. Xiaoyun Li, Nicholas Fallon, and Stephanie Cook assisted with the data collection. Andrej Stancak and Nicholas Fallon provided training on fMRI data analysis. I analysed the data, interpreted the results, and wrote the manuscript. Nicholas Fallon, Stephanie Cook, Timo Giesbrecht, Anna Thomas, Joanne Harrold, Jason Halford, and Andrej Stancak contributed useful comments on the manuscript.

Acknowledgements

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6.1 Abstract

Hunger and pain are powerful homeostatic drives. Manipulations of appetite can produce robust effects on subjective and neural processing of pain. We defined a network of brain areas commonly implicated in pain processing, and investigated appetite-induced changes in the functional connectivity of the network when challenged by moderately painful laser stimuli. Twelve healthy participants underwent two fMRI scans, one after an overnight fast, the other following a large breakfast. Pain trials were discarded if participants had rated them as 'not painful', and those remaining were submitted for analysis in a paired t-test design. Functional connectivity between areas involved in stimulus saliency processing (anterior insula, ACC, and prefrontal cortex) was stronger during fasting, while functional connectivity between default mode structures (PCC and precuneus) was stronger during satiation. The enhanced PCC-precuneus connectivity could be accounted for by differences in subjective hunger ratings between scans. Functional connectivity that likely underlies pain intensity coding, memory, and descending pain modulation was unaffected by changes in the homeostatic energy balance. Fasting appears to boost the saliency of any stimuli, which is likely advantageous when searching for food. Satiety is conducive to default mode activation, even in the face of moderate pain.

6.2 Introduction

The neural basis of pain perception has been widely explored. There are a number of brain areas that have been repeatedly cited as being activated by short painful stimuli, and appear to constitute a widely distributed pain perception and affective evaluation network. Frequently reported components include the thalamus, insula (particularly contralateral to the painful stimulus), ACC, PCC, prefrontal cortex (PFC), amygdala, and hippocampus (Apkarian et al. 2005; Peyron et al. 2000). Pain-related precuneus activation is less clear; some studies show precuneus activation in response to painful stimuli (Maleki et al. 2013; Ter Minassian et al. 2013), others show pain-induced deactivation when precuneus is engaged with other DMN regions (Kong et al. 2010; Mantini et al. 2009). It is included here in the hopes of further elucidating the role of precuneus in pain perception.

The thalamus is a major target of nociceptive fibres originating in the spinothalamic tract (Bastuji et al. 2015; Garland 2012; Hodge and Apkarian 1990). From there, the pain signal is passed on to posterior insula (Dum et al. 2009). The role of thalamus and posterior insula in ascending pain processing appears to be closely related to physical sensation, with both implicated in mapping pain intensity (Coghill et al. 1999; Ostrowsky et al. 2002; Stephani et al. 2011; Wager et al. 2013). From posterior insula, the pain signal continues forward to anterior insula (Frot et al. 2014). Anterior insula is part of a wide affect-related network consisting of (among other areas) the ACC, amygdala, and PFC (Boccard et al. 2014; Derbyshire et al. 1997; Rainville et al. 1997). Hippocampal cortex and PCC may subserve memory-related processing during pain. They share dense connections (Leech and Sharp 2014), and while the specific roles of PCC are not yet well-understood, it is most often cited in memory or pain studies (Nielsen et al. 2005). The vital role of hippocampus in memory is well-established, and hippocampus activation to pain is likely involved in memory of past pain and the stress response to current pain (Liu and Chen 2009; Schwedt et al. 2014).

Pain induced activation of areas of this network can be modulated by many competing variables. Of interest here is one with homeostatic qualities that may compete with pain perception: appetite. Previous studies have shown that hunger is

represented in many of the same areas as pain (Tataranni et al. 1999; van Rijn et al. 2015), and in addition to hunger suppressing behavioural responses to pain, reduced neural activation to nociceptive stimuli during fasting has also been observed (Wright et al. 2015). Since pain itself is a homeostatic drive (Craig 2003b), competition between hunger and pain may modify the strength of functional connections between areas of the pain network.

The current study modelled the pain-induced functional connectivity between the thalamus, anterior and posterior insula, ACC, PCC, amygdala, hippocampus, precuneus, and PFC, and investigated changes in connection strengths induced by fasting and satiation. We hypothesised that painful laser stimuli applied to the dorsum of the foot would compete with fasting-induced hunger, leading to some alterations in functional connectivity between these brain areas. This is the first study to investigate functional connectivity during nociception under conditions of hunger and satiety.

6.3 Methods

6.3.1 Screening

Safety screening was carried out by a radiographer. The laser intensity to be used in the subsequent fMRI sessions was selected during the screening. Painful stimuli were produced using an Nd-YAP laser stimulator (Stim1324, El.En., Italy), with a spot size of 5 mm and a pulse duration of 3 ms. A 5 cm circle was drawn on the dorsal area of the participants' left foot, and laser stimuli applied pseudorandomly within. Stimulus intensity scaling started at 1.5 J, and was incrementally increased in 0.25 J steps until participants gave a rating of 3-4 out of 7 (moderate pain). The scale was anchored as '1: no pain' and '7: worst imaginable pain'. When this rating was achieved, five more stimuli at this intensity were applied to ensure that the rating remained consistent. The scaling was carried out while participants lay on the scanner bed with their head in the head-coil, giving some opportunity to acclimatise to the scanner environment before the experimental sessions.

6.3.2 Participants

Twelve healthy volunteers (six male) with a normal BMI (World Health Organization 2006) from the undergraduate and postgraduate student population of the University of Liverpool took part in this study. The mean age of the participants was 25 ± 4. Participants gave their written informed consent and the study was conducted in accordance with the Declaration of Helsinki. Local ethical approval was obtained from the University of Liverpool Research Ethics Committee.

6.3.3 Procedure

Participants attended two sessions, separated by at least 7 days. For the fasted session, they came for the scan after an overnight fast of at least 9 hours, and were not provided with breakfast. For the fed session, they were given a fixed load breakfast consisting of corn flakes, semi skimmed milk, toast, margarine, jam, and orange juice after the overnight fast, and then completed the scan. The total energy content of the fixed load breakfast was 531 kcal (2223 KJ) for females, and 791 kcal (2779.5 KJ) for males. The order of fed and fasted sessions was counterbalanced across participants.

Hunger was measured using a 100 mm VAS immediately prior to the scans in both sessions. Blood glucose samples were obtained using a handheld monitor (Model: Accu-Chek Aviva, Roche Diagnostics Ltd., West Sussex, UK) immediately after the completion of the VAS. The day before both sessions, participants were instructed not to exercise more than they would normally, and not to eat or drink anything other than water from midnight. Compliance was assessed using diary entries and blood glucose testing. Participants also completed the POMS (McNair et al. 1971) at the start of both sessions.

Before beginning the experiment a 5 cm circle was drawn on the dorsum of the left foot, and laser stimuli were applied pseudorandomly within. The laser intensity used was that which had been selected during screening. Participants were shown how to rate their pain using an MRI button box, and then completed five practice trials. If participants completed these successfully and consistently rated the pain they had felt as moderate (at least 4 on the 7 point scale), the experiment was started immediately. If

participants did not rate the pain as moderate, the laser intensity was increased until they did so consistently, and then the experiment was started. The same stimulus intensity was used for both sessions.

The fMRI experiment was comprised of one continuous block containing 44 trials. Each trial began with a fixation cross, the duration of which was jittered randomly between 9000 and 13,000 ms. The fixation cross was then replaced by a photograph of either food or an object. Photographs were presented in a pseudo randomised order, such that no more than three photographs from the same category were presented consecutively. The photograph was visible for exactly 5000 ms, and a laser stimulus was applied randomly between 1000 and 4000 ms after the onset of the photograph. The screen went blank for 1000 ms, and then participants rated the pain they had felt on the scale of 1-7. A visual representation of the trial structure is shown in Figure 15. E-Prime 2.0 Professional (Psychology Software Tools, Inc: Pennsylvania, USA) was used as the stimulus presentation program. All photographs had a light coloured background and measured 492 x 329 pixels, with a resolution of 72 dpi.

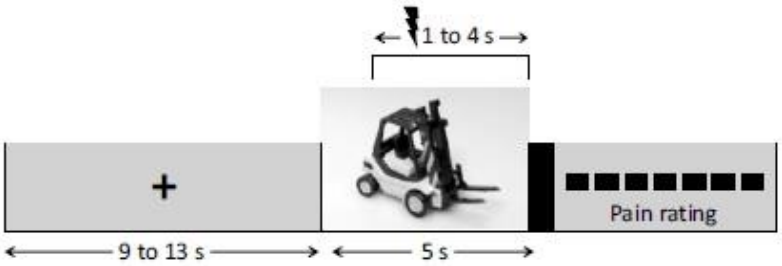

Figure 15 Trial structure. Each trial began with a fixation cross randomly lasting between 9 and 13 s. It was replaced by a photograph of an inedible object, visible for 5 s. A painful laser stimulus was applied randomly between 1 and 4 s after the onset of the photograph. The screen went blank for 1 s, and then participants rated the pain they felt on a scale of 1 to 7 (1 = no pain; 7 = worst imaginable pain).

6.3.4 Data acquisition

Scans were undertaken on a whole-body Siemens Trio 3T scanner (Siemens, Erlangen, Germany) with an eight-channel RF head-coil. Foam padding and cheek clamps were used to restrict head movement. Two dummy scans at the start of the block were discarded, in order to remove T1 saturation effects. An EPI sequence was used to acquire functional images covering the whole brain (42 axial slices), TR = 2500 ms, TE

= 30 ms, slice order = interleaved ascending, flip angle = 90°, matrix = 64 x 64, field of view = 192 mm, slice thickness = 2.5 mm (0.5 cm gap), voxel size at acquisition = 3.0 x 3.0 x 2.5 mm.

Grey matter, white matter, and CSF masks were produced by segmenting the SPM template EPI image in SPM. The thalamus, ACC, PCC, and bilateral amygdala, hippocampus, prefrontal cortex (PFC), and precuneus ROIs were produced using the SPM WFU Pickatlas toolbox (www.nitrc.org/projects/wfu_pickatlas; Maldjian et al. 2003; 2004). The PFC ROIs are composed of the 'frontal superior', 'frontal superior medial', and 'frontal mid' masks. Bilateral anterior insula and posterior insula ROIs are based on co-ordinates from Cauda et al. (2011), converted from Talairach to MNI space using the 'tal2mni' script (Brett 2001). Tissue maps and ROIs were resliced to match the preprocessed functional image dimensions, and imported to Conn. The ROIs are presented in Figure 16 superimposed over a T1 scan from a randomly selected participant.

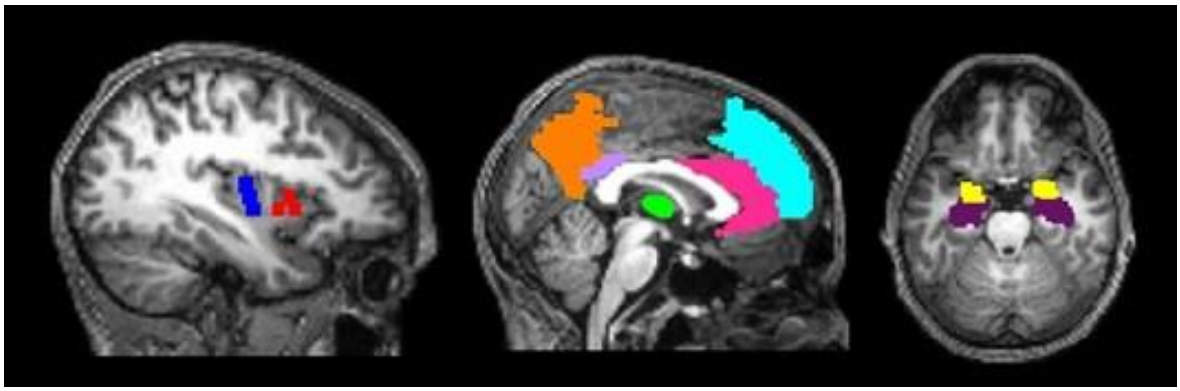

Figure 16 ROIs selected *a priori* for the functional connectivity analysis, superimposed over a randomly selected participant's T1 scan. The ROIs consist of masks created using brain atlas toolboxes compatible with SPM. Red = anterior insula; dark blue = posterior insula; cyan = PFC; pink = ACC; green = thalamus; lilac = PCC; orange = precuneus; yellow = amygdala; purple = hippocampus.

6.3.5 Data rejection

Due to poor neural responsiveness to pain stimuli presented during the food photographs, all trials containing food photographs were dropped from the analysis.

This immediately reduced the proportion of usable data by 50 %. Remaining trials which were given a pain intensity rating of 1 (‘no pain’) were also omitted from the analysis, as we were only interested in brain activity to painful stimuli. Due to the paired t-test design, the maximum number of trials accepted per participant was equal to the minimum number of trials given a pain intensity rating of 2 or above by that participant in either session. The mean number of submitted trials per participant was 18.6 ± 2.2and the total number of trials entered into the analysis was 224; representing 44 % of the overall original number of collected trials.

6.3.6 BOLD data analysis

SPM8 was used to preprocess the data. Images were slice-timing corrected, realigned and unwarped to correct for head motion, normalised to the SPM EPI template, and smoothed with a 6 mm full width half maximum Gaussian kernel. At the first level, trials were defined as beginning at the application of the pain stimuli and lasting for one second. The BOLD responses to pain were evaluated separately for the fasted and fed conditions using one sided t-tests, and the first level results were then entered into the second level analysis in a paired t-test design.

6.3.7 Functional connectivity data analysis

The preprocessed images from the BOLD data analysis were imported to the functional connectivity toolbox Conn v.13 (www.nitrc.org/projects/conn; Whitfield-Gabrieli and Nieto-Castanon 2012).

The entire time course of data was entered into the set-up stage of the analysis. The data were band-pass filtered from 0.008-0.09 Hz to remove noise and low frequency drift, and signal from white matter and CSF was defined as confounds and removed with linear regression. Realignment parameters were entered as first level covariates.

Trials were defined as beginning at the application of the pain stimuli and lasting for one second, and were convolved with a Gaussian kernel to emulate the HRF. They

were entered in separate fasted and fed conditions, in a paired t-test design. At the first level, ROI-to-ROI connectivity maps for each participant and each ROI were generated separately for the fasted and fed sessions. For group-level analysis, one-sided t-tests were used to examine changes in ROI-to-ROI connectivity between sessions. No restrictions were placed on connectivity between any of the ROIs; each ROI was free to be tested in combination with any of the others.

6.3.8 Statistical analyses

Behavioural and blood glucose data were analysed with SPSS v.22 (IBM, NY, USA), using paired Student's t-tests; FDR-corrected functional connectivity maps were derived from Conn's native analyses. Correlational data were extracted from Conn's first level statistics output and analysed in SPSS.

6.4 Results

6.4.1 Behavioural and glycaemic

There were no significant differences in reported mood between the sessions ($p > .05$), and no significant differences in pain ratings between sessions ($p > .05$), or between photograph type in the context of sessions ($p > .05$). Pain ratings for session and photograph type are presented in Table 6 below.

Session		Session + Photograph Type			
Fasted	Fed	Fasted + F	Fasted + O	Fed + F	Fed + O
3.4 ± .7	3.2 ± .7	3.3 ± .8	3.4 ± .9	3.2 ± .7	3.2 ± .7

Table 6 Pain ratings by session, and by photograph type in the context of session. F = food photograph; O = object photograph; ± = standard deviation.

VAS ratings showed that during the fasted session, participants were significantly more hungry than they were during the fed session ($t(11) = 19.1$, $p < .001$). Blood glucose readings were unavailable for two participants; data from the other ten participants showed that scores were significantly lower during the fasted session than during the fed session ($t(9) = -7.5$, $p < .001$).

6.4.2 Subtraction BOLD results

The contrast fasted > fed did not produce any significant results with a FWE corrected threshold, or at $p < .001$ uncorrected . The contrast fed > fasted did not produce any significant results with FWE correction, but produced two significant clusters at $p < .001$ uncorrected with a 100 voxel minimum cluster extent threshold. The first was located in the ACC (MNI coordinates = 0, 30, 30; k = 271; t = 6.1); the second in the left MFG (MNI coordinates = -38, 30, 34; k = 293; t = 7.0).

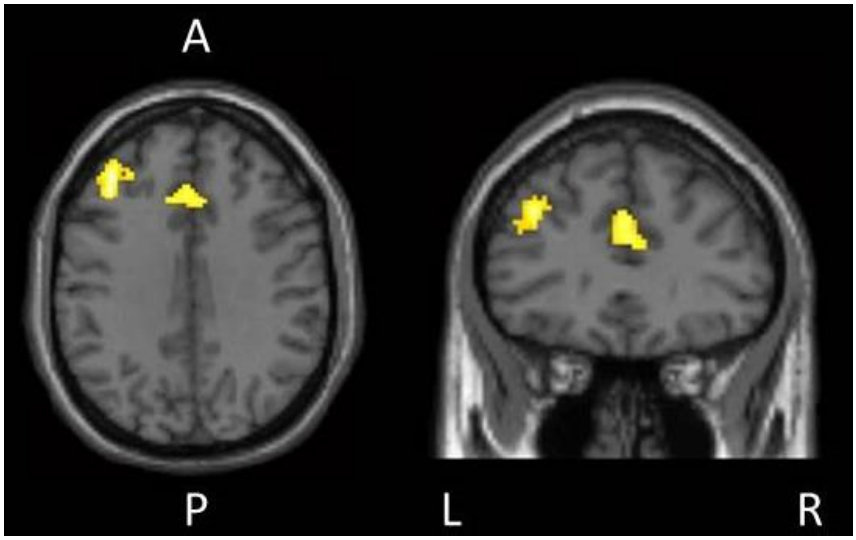

Figure 17 BOLD data analysis results superimposed over the SPM8 EPI template. The contrast fed > fasted produced significant clusters in the ACC and MFG. A = anterior; P = posterior; L = left; R = right.

6.4.3 Functional connectivity results

Functional connectivity results are shown in Figure 18. The underlined ROIs appear to be 'hubs' which are functionally connected to several different areas. Dashed purple lines indicate equal functional connectivity between both sessions (FDR-corrected, p <

.05). A solid red line shows stronger connectivity during the fasted session (contrast: fasted > fed, FDR-corrected, $p < .05$). A solid blue line shows stronger connectivity during the fed session (contrast: fed > fasted, FDR-corrected, $p < .05$).

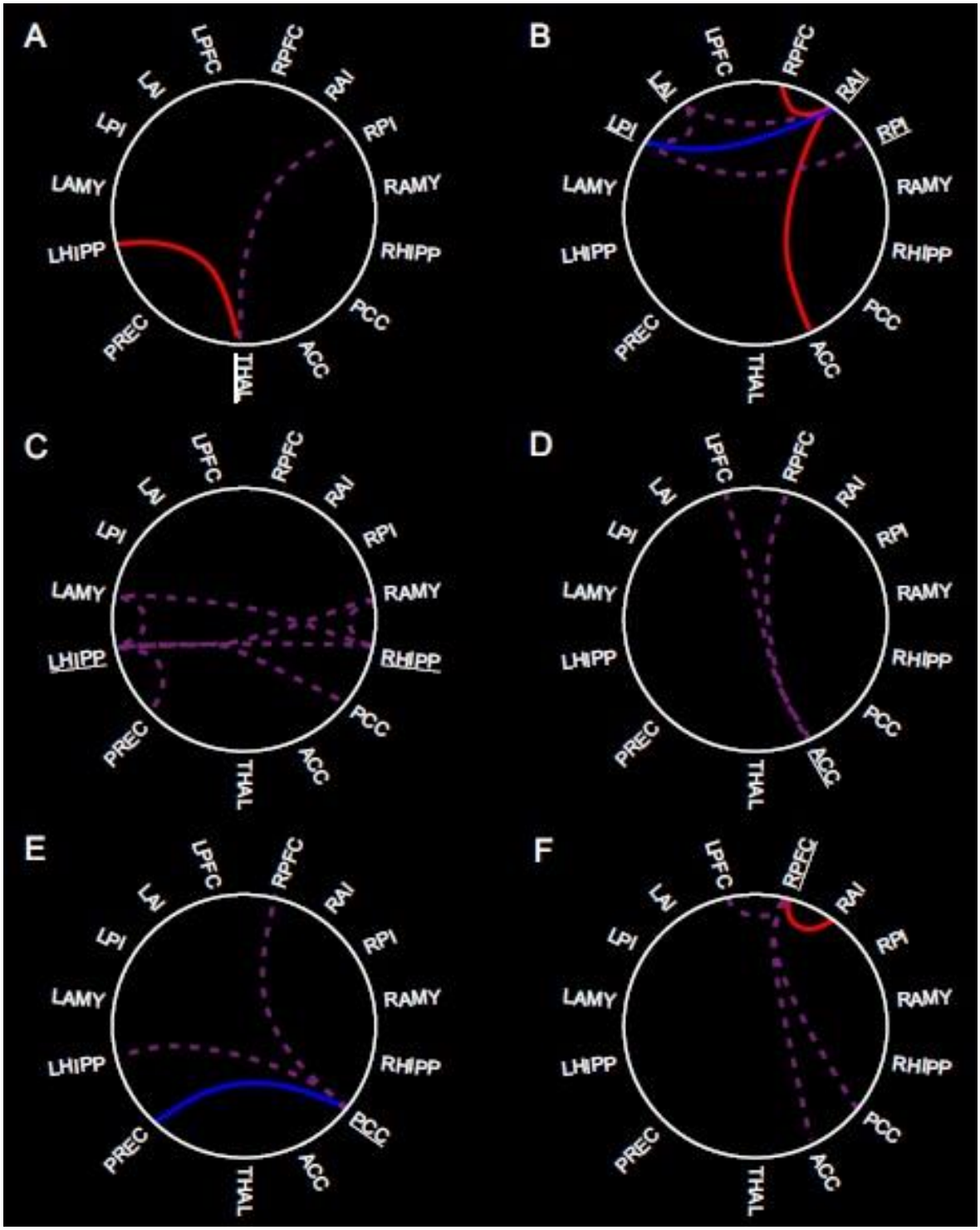

Figure 18 Functional connectivities provoked by painful laser stimuli. Purple dashed lines are connectivities that are equally strong across the fasted and satiated conditions. Solid red lines indicate functional connections that were stronger during the fasted session than the fed session; solid blue lines are functional connectivities that were stronger during the fed session than the fasted session. THAL = thalamus; RHIPP = right hippocampus; LHIPP = left hippocampus; RAMY = right amygdala; LAMY = left amygdala; RPI = right posterior insula; LPI = left posterior insula; RAI = right anterior insula; LAI = left anterior insula; RPFC = right PFC; LPFC = left PFC; PREC = precuneus.

The thalamus ROI is connected to right posterior insula (RPI) equally during both sessions, and significantly more strongly to left hippocampus during the fasted session. Insula cortex is highly interconnected. Right anterior insula (RAI) is significantly more strongly connected to the ACC and right PFC during the fasted session, and to left posterior insula (LPI) during the fed session. The hippocampal ROIs are also densely connected with each other, and with bilateral amygdala. The ACC is connected to bilateral PFC during both sessions; PCC is connected to right PFC and left hippocampus. The PCC is more strongly connected to precuneus during the fed session.

6.4.3 Covariates

The enhanced functional connectivity between PCC and precuneus disappeared when hunger ratings (expressed as Δ difference scores between sessions) were added as a second level covariate, underscoring the association of this connectivity with changes in appetite induced by the experiment paradigm.

6.5 Discussion

No significant activations were seen for the contrast fasted > fed, but fed > fasted produced significant clusters in the ACC and MFG. The ACC is a core component of the pain matrix, cited almost without exception in neuroimaging studies of pain. The MFG is also often cited in pain studies (e.g. Baron et al. 1999; Brooks et al. 2002; Iadarola et al. 1998; Song et al. 2006). The appearance of a significant cluster in MFG did not constitute a valid reason to exclude the rest of the PFC from the functional connectivity analysis, since other areas of frontal cortex are frequently found to be

active during the experience of pain (Bornhovd et al. 2002; Derbyshire et al. 1997; Ohara et al. 2004a; Seifert et al. 2012). There may also have been areas responding to pain equally strongly in both fasted and satiated conditions that the simple subtraction analysis could not identify (Sommer 2002). Nonetheless, these results provided some support for our *a priori* selection of ROIs for the functional connectivity analysis, and some reassurance that the collected data was sufficient despite having to discard over 50 % of the trials.

The functional connectivity results showed that appetite manipulations provoked some significant changes in connectivity between areas of the pain network; connectivity between other areas remained unaffected.

6.5.1 Altered connectivity during fasting

Lateralised (Almashaikhi et al. 2014; Augustine 1996; Cloutman et al. 2012) and cross-hemispheric (Gay et al. 2014) insula subregions are highly interconnected in humans. Anterior insula has previously been shown to be part of a salience network which also contains the ACC (Cauda et al. 2012; Fox et al. 2006; Kemmer et al. 2015), and is often also active in conjunction with the PFC (Menon and Uddin 2010; Seeley et al. 2007; Sridharan et al. 2008; Uddin 2015). It appears to function as a 'hub', connecting salient stimuli with higher order processing, interoceptive, and affect-related areas (Critchley et al. 2004; Menon and Uddin 2010; Peltz et al. 2011; Pessoa 2013; Sterzer and Kleinschmidt 2010; Uddin 2015). The enhanced functional connectivity during fasting between RAI and ACC, and RAI and right PFC (Figure 18, panel B) is not due to heightened pain perception, since pain ratings did not differ across sessions. Rather, these results seem to be due to enhanced saliency of any stimuli when appetite is heightened. Other studies have reported similar intensification of physiological responses to both food-related and food-unrelated stimuli during hunger (Loch et al. 2015; Tong et al. 2011; Wright et al. 2015), which is presumably advantageous when seeking food (Stafford and Welbeck 2011).

The thalamus is structurally connected to hippocampus (Xia et al. 2012), a limbic structure which is involved in anxiety and catastrophising about pain (Gondo et

al. 2012; Lin et al. 2013; Ploghaus et al. 2001). Again, the enhanced connectivity found during fasting (Figure 18, panel A) may be due to a general effect of enhanced stimulus saliency when appetite is heightened. Hippocampal and thalamic activation has been shown to be related to stimulus saliency in other contexts (Deborah et al. 2002; Kushnir et al. 2013; Zweynert et al. 2011).

6.5.2 Altered connectivity during satiation

The increased functional connectivity between left posterior and right anterior insula seeds during the fed session (Figure 18, panel B) may be stable and attributable to satiety rather than anything to do with pain. A previous study using water deprivation instead of fasting found that bilateral anterior insula and left posterior insula are part of a network which shows increased functional connectivity after thirst satiation (Farrell et al. 2011); possibly a similar network is activated by hunger satiation.

The PCC was more strongly connected to precuneus during satiety, and this enhanced connectivity was eliminated when Δ hunger scores were added as a second level covariate, underscoring the association with the appetite manipulation. Precuneus and PCC are core parts of the DMN (Fransson and Marrelec 2008; Laird et al. 2009; van den Heuvel et al. 2008). While it seems counterintuitive that the DMN could be active in a study utilising painful stimuli, other research has shown that participants' attention does spontaneously 'wander' while noxious stimuli are applied, and that these lapses of attention are associated with DMN activity (Kucyi et al. 2013). The relationship between metabolic energy balance and the DMN is under-explored, and what little research exists focusses predominantly on obesity (e.g. Kullmann et al. 2012; McFadden et al. 2013; Tregellas et al. 2011). However, previous research from our laboratory with healthy participants found increased functional connectivity between posterior insula and DMN areas during satiety in a recent resting-state study (Wright et al. 2016). The DMN activation during satiety may be due to feeling restful after eating a large meal; neurons which prompt wakefulness and food seeking during fasting are switched off by glucose administration (Burdakov et al. 2005).

6.5.3 Unaltered connectivity

Connectivity between thalamus and contralateral posterior insula (RPI) is equally strong during both sessions (Figure 18, panel A). It is likely that this is a bottom-up pathway; there are ascending nociceptive connections between brainstem and thalamus (Brooks and Tracey 2005), and posterior insula receives direct projections from spinothalamic tract neurons (Dum et al. 2009). Findings from electrophysiological studies also describe pain-induced activation of posterior insula before anterior insula (Frot et al. 2014). Thalamus (Coghill et al. 1999; Wager et al. 2013) and posterior insula (Ostrowsky et al. 2002; Stephani et al. 2011; Wager et al. 2013) appear to map pain intensity. Since pain ratings were not significantly different across sessions, a difference in functional connectivity at this point would be unexpected.

The hippocampus and amygdala (Figure 18, panel C) are structurally, functionally, and effectively connected (Fastenrath et al. 2014; Liu et al. 2011; 2015; Zhou et al. 2015), and the functional connectivity between them is enhanced by stress (Ghosh et al. 2013; Vaisvaser et al. 2013). Previous observations of amygdala and hippocampal activation by pain stimuli have been attributed to emotional learning (Liu et al. 2010), and vigilance to upcoming pain stimuli (Liu et al. 2011). Experiencing more generally aversive events such as financial loss (Hahn et al. 2010) also appears to evoke increases in amygdala-hippocampal functional connectivity, as does memory retrieval of emotionally valenced information (Smith et al. 2006), and memory formation under the threat of punishment (Murty et al. 2012). In the current study pain was rated as equally intense across sessions, and therefore likely also experienced as equally aversive. The lack of differences in amygdala-hypothalamic functional connectivity strengths is therefore to be expected, if the connectivity is indeed due to the aversiveness of the pain stimuli. However, hippocampus is also functionally connected to precuneus and PCC in the current study. These areas are structurally connected (Palesi et al. 2012; Parvizi et al. 2006; Teipel et al. 2010), and the addition of PCC-precuneus functional connectivity to amygdala-hippocampal connectivity has previously been found following acute stress (Veer et al. 2011). This network seems ideally placed to integrate pain-induced affect with autobiographical memory; we requested a pain rating after every trial, and negative arousal may form an important cue when comparing the perceived intensity of the nociceptive stimulus to previous painful

stimuli. It is not possible to determine which, or even if either, of these explanations is correct, but both would account for the equal strengths of functional connectivity across sessions.

Cingulate cortex (ACC and PCC) was equally functionally connected to PFC during both sessions (Figure 18, panels D, E, F). Cingulo-frontal connectivity may be a pathway for descending pain modulation (Valet et al. 2004; Watson et al. 2009), possibly by the top-down modulation of negative affective response to pain (Kong et al. 2013), though it should be noted that several studies implicating the ACC in placebo analgesia did not find a similar role for PCC (Kong et al. 2013; Koyama et al. 2005; Price et al. 2007; Wager et al. 2004). If descending pain modulation is indeed dependent on reduction of negative emotional responses then it would make sense that the ACC has a role to play while the PCC does not. However, this relationship is not yet established, and will likely become clearer as other network analyses are conducted. Regardless, the subjective pain ratings being equal across fasting and satiety would be expected if the cingulo-frontal connectivity observed here is due to descending pain modulation.

6.5.4 Comparison of subtraction and functional connectivity results

At first glance it appears that the subtraction and functional connectivity results are conflicting, but this is actually not the case. The subtraction analysis produces information concerning differences in the degree of activation of discrete areas across conditions; the functional connectivity analysis is not concerned with the degree of activation of discrete areas and only discerns shared patterns of activation between areas. Functional connectivity is calculated from the correlation between *time series* of different brain areas (Friston 1994; Friston et al. 1996). While the ACC and left MFG are more strongly activated in the fed condition, neither area shows stronger functional connectivity in the fed condition, and they are not more strongly connected with each other during the fed session (left MFG is part of the left PFC ROI). So, although the ACC and MFG are more strongly activated during the fed session, their temporal patterns of response to the pain stimuli are equivalent whether the participants are fasted or fed. As mentioned in section 6.5.3, their shared connectivity may be due to their roles

in descending pain modulation. It should also be noted that the clusters identified in the subtraction analysis are dwarfed by the volume of the ROIs used for the functional analysis, which were created from masks defined by brain atlases. The ROIs therefore contain signal from many more voxels than the identified clusters, meaning that the two analyses are actually incorporating different brain areas, especially the MFG.

6.5.5 Limitations

Due to the unforeseen issue of poor neural responsiveness to pain stimuli presented concurrently with food photographs, all trials containing food photographs had to be dropped from the analysis. This removed half the number of trials immediately, and remaining trials given a pain intensity rating of no pain also had to be removed from the analysis. Trial numbers were reduced further due to the paired t-test design employed, meaning that an omission of a trial rated as not painful in one session also had to be removed from the other session. This drastically cut the number of useable trials, and our results should therefore be treated with ample caution.

Another issue is the large seed defined as PFC in the current study. While it is clear from other research that there are several functionally divergent areas within PFC, we had no clear *a priori* motive to pick one, and including them all as separate seeds would have made an already complex study too vast. It would have been more enlightening to subdivide PFC into its various components, but unfortunately this was beyond the scope of this highly exploratory study.

6.5.6 Conclusion

Experiencing moderately painful laser stimuli provokes many functional connections between widely distributed brain regions. Fasting appears to boost the saliency of any stimuli, a result which is in accordance with other studies using food-related and food-unrelated stimuli, and is underlined by the recruitment of nodes of the saliency network: anterior insula, ACC, and PFC. A general increase in alertness is presumably advantageous when searching for food.

Chapter 7

The effect of olfactory stimuli and energy manipulations on nociception

This experiment investigated the effects of a food odour and a pleasant, non-food odour on weak and strong pain during fasting and satiety.

It was written up for submission to Chemical Senses. The format, but not the content, has been altered to match the style of the thesis.

The roles of the co-authors are summarised below:

I designed the study in collaboration with Andrej Stancak and Joanne Harrold. Andrej Stancak, Nicholas Fallon, and Stephanie Cook provided training and assistance with the experiment set-up. I collected and analysed the data, and wrote the manuscript. Andrej Stancak, Nicholas Fallon, Stephanie Cook, Timo Giesbrecht, Anna Thomas, Joanne Harrold, and Jason Halford contributed useful comments on the manuscript.

Acknowledgements

We are grateful to Vicente Soto and Katerina Kokmotou for their substantial assistance with the odour selections.

7.1 Abstract

Hunger and pain are vital homeostatic drives which may compete for attention when experienced simultaneously. While there are contradictory results regarding analgesia induced by manipulations of the homeostatic energy balance, food cues that signal the immediate availability of food appear to consistently disrupt pain processing. We used a bread odour as a salient food cue, and compared pain intensity ratings of electric shock stimuli paired with the bread odour to those paired with a linen odour, and those in a clean air control condition. We hypothesised that the food odour would suppress pain processing more successfully than the linen odour or clean air, especially when participants were in a state of depleted homeostatic energy balance.

Sixteen healthy participants took part in two sessions, one fasted and one satiated. We found that weak pain stimuli were rated as less intense when participants were fasted, regardless of the odour paired with the stimuli. Strong pain stimuli were also rated as less intense when participants were fasted, but only when paired with the bread odour. Our results suggest that food odours may capture attention more than other pleasant but non-food odours, especially when the homeostatic energy balance is depleted.

7.2 Introduction

Pain is a homeostatic drive (Craig 2003b; 2013), and as such, it is logical to speculate that other homeostatic drives could compete with pain for attention. In support of this theory, a plethora of research demonstrates that in humans, ingesting (Blass and Hoffmeyer 1991; Bucher et al. 1995; Zmarzty et al. 1997), or viewing (Wright et al. 2015), palatable substances, experiencing hunger for air (Morelot-Panzini et al. 2007; Nishino et al. 2008; Yashiro et al. 2011), and activation of the sympathetic nervous system by stress (Fechir et al. 2009) can decrease pain perception.

The experience of pain can also be moderated by non-homeostatic-related distractions (e.g. Asl Aminabadi et al. 2012; Cerne et al. 2015; Frankenstein et al. 2001; Jeffs et al. 2014; Ozdemir and Tufekci 2012; Rutter et al. 2009; Sparks 2001; Villarreal et al. 2012) Whether homeostatic or non-homeostatic stimuli are more effective at decreasing pain perception is unclear, and may depend upon the objective pain intensity, pre-existing perturbations of the homeostatic energy balance, or both.

Olfactory stimuli are not frequently used in studies, probably due to the technical challenges involved. Studies with rodents have generally used lemon oil as the odourant. Presentation of lemon oil aroma significantly reduces pain-related behaviour and modulates pain-induced neurochemical release in a variety of brain structures (Aloisi et al. 2002; Ceccarelli et al. 2002; Ikeda et al. 2014). However, it is not at all clear that lemon oil represents an appetitive food odourant (lemons are edible, but it is not wise to take a bite out of one), and therefore it is doubtful that it is immediately engaging to homeostatic processes. Rather, lemon oil aroma appears to affect limbic structures; destroying the ACC abolishes the aroma-induced suppression of pain-related behaviour and modulation of pain-induced neurochemical release (Ikeda et al. 2014). One study utilised an odour clearly related to homeostasis, a predator odour, and found that predator odour stress produced hyperalgesia to heat pain (Roltsch et al. 2014).

In humans, there is a similar dearth of information regarding homeostatically relevant odours. One study found that odours judged to be unpleasant had the effect of increasing pain perception (Bartolo et al. 2013), though other investigators did not observe this effect (Marchand and Arsenault 2002). One more study concluded that the valence of the odour was unimportant; both pleasant and unpleasant odours resulted in

heightened pain in comparison to no odour (Martin 2006). The majority of research, though, has found that pleasant odours reduce the perception of pain (Aou et al. 2005; Bartolo et al. 2013; Demers et al. 2004). This occurs even if participants are not consciously aware of any odour manipulation taking place (Leduc et al. 2007).

The majority of human research with food odours and pain has focussed on infants. Interestingly, in studies that controlled odour familiarity by having very young infants as the participants, familiar odours (mother's milk, vanilla) led to decreased pain-induced behaviours during the time after a painful procedure; pleasant but unfamiliar odours did not (Goubet et al. 2003; Rattaz et al. 2005). Other research showing that infants exhibited fewer pain-induced behaviours after smelling their mother's milk in comparison to infants smelling a different mother's milk or formula milk (Nishitani et al. 2009) may, therefore, not actually have found a specific anti-nociceptive effect of the infants' own mother's milk so much as a more general effect of odour familiarity.

Only two studies that used overtly homeostatically relevant odours and adult participants could be located. One (Villemure and Bushnell 2007) presented androstadienone, a pheromone found in men's sweat, and found that perceived pain intensity was increased, particularly in women. The authors hypothesise that this effect was due to the compound heightening participants' attention. The other study (Prescott and Wilkie 2007) compared the effect of a sweet smelling odour (caramel) and a pleasant odour that did not smell sweet (aftershave) on pain tolerance. Despite the odours being rated as equally pleasant, the sweet odour significantly increased pain tolerance, while the pleasant, non-sweet odour did not affect pain tolerance. The authors interpret these results as being due to a conditioned association between sweet odours and sweet tastes.

7.2.1 Food odours

Responses to food odours differ from responses to non-food odours, and this modulation varies according to satiety status; after consuming a meal, participants reported a decrease in the pleasantness of food odours (alliesthesia) but no decrease in

response to non-food odours (Albrecht et al. 2009; Duclaux et al. 1973). Some neurons in monkey OFC have been found to decrease their firing rate in response to the smell of a food fed to satiety, while retaining or increasing their firing rate in response to the smell of other food and non-food odours (Critchley and Rolls 1996). Decreased activation has also been found in human OFC in response to the smell of a food (banana) eaten to satiety, with no such decrease in response to another food odour (O'Doherty et al. 2000). One additional study found that insula showed activations specific to food odour (Small et al. 2007). Taken together, these studies suggest that food odours hold a special significance in comparison to other biologically irrelevant odours, and may therefore interfere with pain processing.

7.2.2 Aims and hypotheses

If appetite and pain do indeed compete for attention and a behavioural response, as suggested by previous studies (e.g. LaGraize et al. 2004; Mason and Foo 2009; Wright et al. 2015), food odour could interfere with pain processing so that pain intensity and / or pain unpleasantness are attenuated. These effects may be stronger when participants have been fasting overnight and are in a state of homeostatic energy deficit.

In order to assess whether food odours are indeed the only odours capable of modulating pain perception, we compared the effects of a food odour and a non-food odour that were closely matched in terms of intensity, pleasantness, and familiarity. We decided not to use an unpleasant odour as some other studies have done, as the food odour is supposed to evoke participants' appetite, a process which could easily be disrupted by a disgusting unpalatable odour. Previous studies using odours to evoke appetite also opted to omit unpleasant odours from their design (Ramaekers et al. 2014).

Lastly, the effects of odours may be different across different pain intensities, as strong pain clearly has more homeostatic significance than weak pain. We therefore designed an experiment where strong or weak pain stimuli were presented during pulses of food odour, non-food odour, or clean air, and asked participants to rate pain intensity, pain unpleasantness, and odour intensity after each odour pulse. The experiment was repeated twice, once after an overnight fast, and once after breakfast. We predicted that

the food odour would suppress pain processing more than the non-food odour, especially during a fasting-induced energy deficit.

7.3 Methods

7.3.1 Participants

People with asthma or an eating disorder were not permitted to take part in the study. Potential participants were screened for olfactory acuity using Sniffin'Sticks (US Neurologicals LLC, Washington, US); only people with a score of 75 % or above were accepted. Sixteen healthy participants (4 male, 12 female) from the undergraduate and postgraduate student population of the University of Liverpool took part in this study. The average age of the participants was 27 years ± 7 (mean ± SD). Participants gave their written informed consent, and the study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the University of Liverpool Research Ethics Committee.

7.3.2 Procedure

The food odour used in the experiment was bread (Symrise Ltd., Netherlands); the pleasant non-food odour was fresh linen (Mistral Chemicals, UK). Both were diluted in propylene-glycol (1, 2-Propanediol 99%, Sigma-Aldrich Ltd., UK). The intensity of the bread and linen odours used were individually scaled for participants during the preliminary screening session. They were matched closely for pleasantness, familiarity, and intensity, while the bread odour had to be rated as smelling definitively 'edible', and the linen odour rated as definitely not edible. These odour intensities were then used for the experimental sessions.

Participants were asked not to eat or drink anything other than water from midnight before they attended the experiment at 9 am. Compliance was assessed with diary entries and blood glucose testing. Participants completed two sessions, 23 ± 24 days apart. They either remained fasted for the experiment (fasted session), or were provided with a fixed-load breakfast (fed session). The breakfast consisted of

cornflakes, semi-skimmed milk, a cereal bar, and apple juice, and contained approximately 21 % of the recommended daily calorie allowance for both males and females. Measures of hunger and blood glucose were taken immediately prior to the experiment in both sessions.

The experiment took place in a well-lit, sound attenuated room. Participants wore a respiration belt (ADInstruments Ltd., Oxford, UK) around the epigastrium to allow the experimenter to trigger the olfactory stimulus release at the point of inspiration. Olfactory stimuli were delivered approximately 1 cm under the participants' nose using a custom-built olfactometer (DancerDesign, Wirral, UK). Odours were embedded within a constant clean air flow of approximately 2 litres / minute, in order to avoid any effects of participants sensing changes in the strength of the air flow (Huart et al. 2012). A BlueAir 203 air purifier (BlueAir Ltd., Sweden) was run at maximum power throughout to minimise residual odour from previous trials.

Pain stimuli were produced using electric shocks. A train of three electrical stimuli separated by 15 ms were delivered with two electrodes (cathode proximal; anode distal) attached to the little finger, produced by a Digitimer^{TM} electrical stimulator. The temporal closeness of the stimuli in the train produced a subjective feeling of one electric shock.

Three odour types were presented: bread, linen, and clean air (control odour). A pain stimulus, either strong or weak, was embedded within each odour pulse. This design produced six trial types: bread + strong pain, bread + weak pain, linen + strong pain, linen + weak pain, clean air + strong pain, and clean air + weak pain. The experiment consisted of 90 trials, split into three blocks, in order to allow participants to take a short break and relax in between. Each block contained 5 of each trial type, presented pseudo-randomly. The pain stimuli were individually scaled for each participant at the start of the first experimental session, with an intensity of three out of ten utilised as the weak stimulus, and seven out of ten as the strong stimulus. The stimulus intensities used in the first session were used again in the second session. Before the experiment in both sessions, participants rated the odours for familiarity, intensity, and liking. Trial structure is presented in Figure 19.

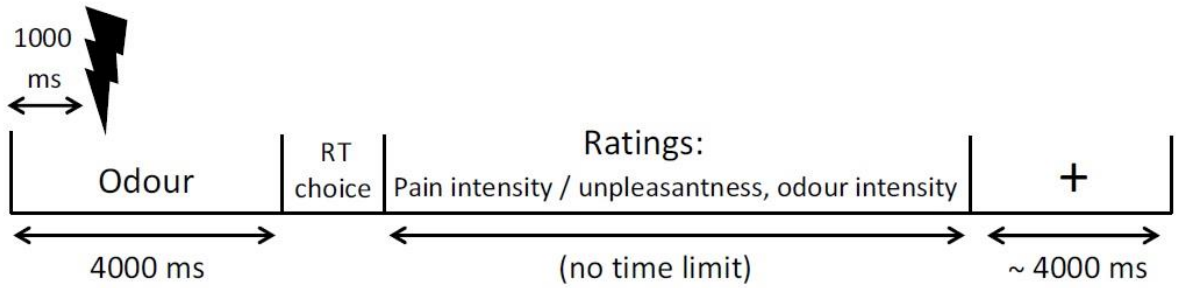

Figure 19 Trial structure. A pseudo-randomly chosen weak or strong pain stimulus was applied within a bread, linen, or clean air odour pulse. Participants were then prompted to indicate whether the pain had been weak or strong, and rated pain intensity, pain unpleasantness, and odour intensity with VAS. After around 4 seconds, the next odour was released when participants started to inhale.

Each trial began with a white fixation cross on a black background. When participants started to inhale, the experimenter manually triggered the odour release. The cross turned blue during the odour presentation, which lasted four seconds. Exactly one second after the start of the odour presentation, an electrical stimulus was applied. When the odour pulse was switched off, the clean air was switched back on, the cross disappeared for one second, and then a forced-choice screen was presented where participants had to decide whether the preceding pain was strong or weak. Lastly, a series of three 100 mm VAS were presented. The first was to rate the pain intensity (anchored with 'no pain' and 'extreme pain'), the second to rate pain unpleasantness (anchored with 'neutral' and 'extremely unpleasant'), and the third to rate odour intensity (anchored with 'no odour' and 'very intense odour'). There was a gap of at least 15 seconds between odour pulses.

E-prime 2.0 professional (PST, Pittsburgh, USA) was used to present odour and pain stimuli. LabChart 7 (ADInstruments, Oxford, UK) was used to monitor respiration data.

7.3.3 Statistical analysis

SPSS v. 20 (IBM) was used to perform statistical analyses. Trials with clean air as the odour stimuli were removed if participants rated the odour intensity as 20 % or higher;

these trials were likely contaminated with residual odour from the previous trial and were therefore unsuitable for use as control trials.

7.4 Results

Technical difficulties resulted in missing glycaemia data for one participant in the fasted session, and two participants in the fed session. Since participants showed considerable habituation to the pain stimuli, a common problem which we have experienced previously (Wright et al. 2015), the results described hereafter are derived from only the first block of each session, decomposed into single trials. All results are adjusted with the Holm-Bonferroni correction for multiple comparisons (Gaetano 2013; Holm 1979).

7.4.1 Odour judgements

Familiarity, liking, and intensity ratings were acquired for the bread and linen odours at the beginning of each session. Mean ratings and standard deviations are presented in Table 7.

Table 7

	Fasted			Fed		
Odour	Intensity	Familiarity	Liking	Intensity	Familiarity	Liking
Bread	73 ± 15	82 ± 14	70 ± 13	81 ± 8	85 ± 16	74 ± 14
Linen	81 ± 12	90 ± 9	79 ± 14	88 ± 9	91 ± 5	86 ± 11

There was a main effect of odour across judgement ratings; the linen odour was rated as more intense ($F(1,15) = 18.64$, $p < .01$), familiar ($F(1,15) = 8.87$, $p < .05$), and liked ($F(1,15) = 8.05$, $p < .05$) than the bread odour. There was also a main effect of session across odour types; both odours were rated as more intense ($F(1,15) = 9.37$, $p < .05$) and more liked ($F(1,15) = 5.67$, $p < .05$) during the fed session. There were no significant interactions.

7.4.2 Hunger and glycaemia

Participants were significantly more hungry during the fasted session than the fed session (mean increase of 56 ± 26 on the VAS; $t(15) = 8.64$, $p < .001$), and recorded significantly lower blood glucose levels (mean fasted reading = 5.2 mmol/L ± 0.6 mmol/L; mean fed reading = 7.1 ± 0.9 mmol/L; $t(12) = 8.62$, $p < .001$).

7.4.3 Pain ratings

For weak pain trials there was a main effect of odour across sessions ($F(2,114) = 3.36$, $p < .05$), but no post hoc t-tests were significant after correcting for multiple comparisons. There was also a main effect of session ($F(1,57) = 6.5$, $p < .05$) whereby stimuli were rated as less painful during the fasted session. Post hoc t-tests, corrected for multiple comparisons, showed significant results for all three odours: bread ($t(73) = -2.89$, $p = .02$); linen ($t(78) = -2.18$, $p = .04$); and clean air ($t(58) = -2.34$, $p = .04$). There was no significant interaction.

For strong pain trials there was a main effect of session ($F(1,45) = 6.46$, $p < .05$). There was no main effect of odour and no significant interaction. Post hoc t tests corrected for multiple comparisons revealed that only the bread odour was associated with lower pain ratings in the fasted session ($t(68) = -2.85$, $p = .02$).

Pain intensity ratings by session, stimulus strength, and odour type are shown in Figure 20. Pain unpleasantness ratings were unaffected by satiety status or odour type after correction for multiple comparisons. Very few mistakes were made in identifying the pain stimuli as weak or strong, and there were no significant differences in the number of errors made across session or odour type ($p > .05$).

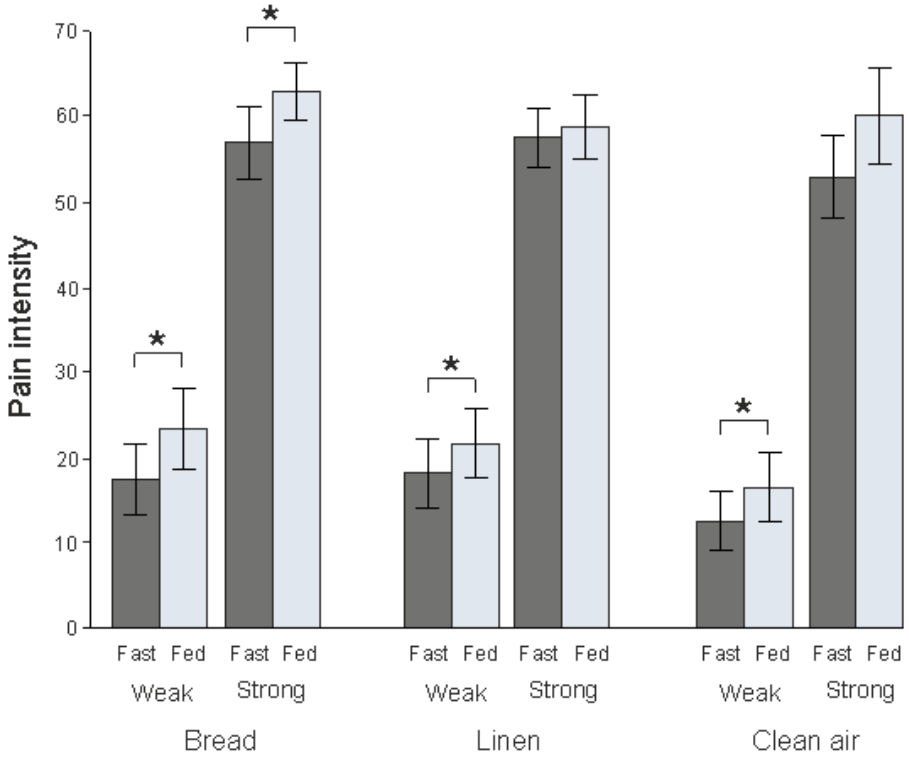

Figure 20 Pain intensity ratings on 100 point VAS categorised by satiety status, pain stimuli strength, and odour. Error bars represent the standard deviations. * = $p < .05$, Holm-Bonferroni corrected.

7.5 Discussion

The effects of odours were different across different pain intensities. Weak pain stimuli across all three bread, linen, and clean air odour conditions were rated as less painful during the fasted session than the fed session, indicating that perceived pain was modulated by appetite regardless of the accompanying odour. Strong pain stimuli were also rated as less painful when participants were fasted, but only when the pain was presented in conjunction with the bread odour. This suggests that food odours may capture attention more than other pleasant but non-food odours, especially when the homeostatic energy balance is depleted, and this may be account for the interference with strong pain processing during fasting. This result extends the findings of Prescott and Wilkie (2007), that a sweet odour (caramel) can increase pain tolerance over and above a pleasant non-food odour.

These results fit into the larger picture of previously published research. Animal and human research on satiety and pain has produced mixed results. Some found an analgesic effect of fasting (Davidson et al. 1992; de los Santos-Arteaga et al. 2003;

McGivern et al. 1979; Michalsen 2010; Wright et al. 2015); while others showed a reduction in pain after feeding (Blass et al. 1987; Haouari et al. 1995; Zmarzty et al. 1997). There are also papers showing increased pain following fasting (Khasar et al. 2003; Pollatos et al. 2012). The most consistent analgesic effects appear to occur when there is a perceived or actual immediacy to food cues. Food odours exert a significant suppression on pain perception (Nakama-Kitamura 2014; Prescott and Wilkie 2007), as does expecting (Dum and Herz 1984), or working for food rewards (LaGraize et al. 2004). The application of pain when eating is already in progress does little or nothing to interrupt ingestion or food gathering (Aloisi and Carli 1996; Foo et al. 2009; Foo and Mason 2005; 2009; Mason and Foo 2009). Our results suggest that when there is a deficit in the homeostatic energy balance in combination with strong pain (both highly salient motivational drives), the presentation of a food cue that signals its' immediate availability significantly inhibits pain perception.

These findings point to a potential use for background food odours in environments where short-duration pain is likely to occur. Previous studies have established that pleasant odours presented ambiently in dental surgery waiting rooms can reduce patient anxiety (Kritsidima et al. 2010; Lehrner et al. 2000, 2005; Zabirunnisa et al. 2014) and cortisol secretion (Jafarzadeh et al. 2013), and some encouraging aromatherapy-related reductions in pain intensity have been recently reported (Ayan et al. 2013; Bagheri-Nesami et al. 2014; Kim et al. 2011). Possibly, food odours in such a situation could produce a reduction in pain perception superior to that produced by pleasant non-food odours. This remains to be ascertained.

7.5.1 Limitations

We set out to match the bread and linen odours for intensity, familiarity, and liking as closely as possible for each participant at the screening session, several days or weeks before they actually took part in the study. This was done in order to ensure that the only salient difference between the odours was their 'edibility' factor. While the ratings acquired at the start of each session show that the odours are not perfectly matched on any of these dimensions (Table 7), the differences between mean ratings are only in the order of 6-12%. It is therefore unlikely, but not impossible, that relatively minor

fluctuations in odour perception between sessions could account for the significant changes in pain intensity perception.

Secondly, pain ratings and odour ratings only remained consistent during the first block; after this the pain ratings began to drop and there was wide variation in odour ratings. Both are likely due to habituation, a common hazard in pain and olfaction research (Gottfried et al. 2002; Weiss et al. 1997). As a result, the number of trials suitable for use was considerably lower than we would have wanted. Our results require replication, and should be treated with caution.

7.5.2 Conclusion

The results of the current study demonstrate that both hunger and food odours suppress subjective pain perception. Short, painful medical procedures may be perceived as less painful if conducted in the presence of an ambient food odour.

Chapter 8

General Discussion

The overall aim of this thesis was to examine behavioural and neural effects of appetite on pain, in an attempt to shed more light on the encouraging but sometimes contradictory results from animal and human studies. It was anticipated that fasting or appetitive stimuli may be useful as benign adjuncts to the standard pharmacotherapy treatment of chronic pain.

In Chapter One, the results of a comprehensive literature review were reported. They divulge only one sure finding – that manipulations of the homeostatic energy balance do indeed have an effect on pain. Whether that effect is inhibitory or potentiating is challenging to predict, due likely to the vast array of study designs encompassing the gamut of fasting times, pain type, and pain severity. Nevertheless, there are some commonalities which should be discussed. A number of the studies reported, whether the analgesia was produced by fasting or feeding, that the opioid antagonists naloxone and naltrexone blocked the analgesia. This strongly suggests that one avenue for fasting or feeding induced analgesia is the endogenous opioid system. Another potential mechanism is the vagal nerve, different afferents of which are activated during fasting or feeding. In support of this theory, artificially stimulating different vagal afferents produces either pro-nociceptive or analgesic results.

Additionally some of the known physical connections between nodes of the pain, hunger, taste, and olfaction pathways were presented, revealing a number of potential brain areas that could underlie a moderating effect of appetite / appetitive stimuli on pain. Areas that stood out as being of particular interest, due to their common involvement in two or more of the pathways, included thalamus, the relay station through which pain, hunger, and taste signals pass; hypothalamus, a hub of all four pathways and a crucial area for both eating and terminating feeding; anterior insula, a target of the pain, taste, and olfaction pathways; amygdala, a limbic area carrying afferent and efferent pain projections, and a region of the hunger and olfaction

pathways; and the ACC, another limbic region with a role to play in descending pain modulation, and a node of the hunger and olfaction pathways. Taken together with the results of the literature review, this provides a solid justification for the investigation of the effects of appetite / appetitive stimuli on pain.

8.1 Summary of findings

It was first necessary to check that an overnight fast of around 9 hours was sufficient to induce a significant drop in blood glucose, and to influence neural activity. This timeframe was chosen to cause minimum inconvenience to the participants, since they would be fasting while asleep anyway. The first two experimental chapters (Chapters 3 and 4) used a simple paradigm, whereby participants attended two resting state fMRI scans. One took place after an overnight fast, the other after breakfast. Blood glucose measurements were acquired before each scan. It was hypothesised that blood glucose manipulations would induce alterations of functional connectivity in areas crucial to the homeostatic energy balance. We found that an overnight fast was indeed capable of producing a significant decrease in blood glucose levels, and alterations of resting state functional connectivity.

In Chapter 3, we took left and right anterior, middle, and posterior insula seeds, and mapped changes in their functional connectivity during conditions of fasting and satiety. We found that during fasting, functional connectivity between the left posterior insula and cerebellum / superior frontal gyrus was stronger. During satiety, functional connectivity was stronger between the right middle insula and some default mode structures, the left and right posterior parietal cortex and PCC. The alterations in functional connectivity between the left posterior insula and superior frontal gyrus during fasting could be accounted for by differences in blood glucose levels between the scans.

In Chapter 4 we took left and right hypothalamus as seeds and mapped changes in their functional connectivity under the same conditions. During fasting, functional connectivity between left hypothalamus and right inferior frontal gyrus was enhanced. During satiety, there was enhanced functional connectivity between right hypothalamus

and superior parietal cortex. Both connectivities appear to be related to cognitive control over eating.

Having confirmed that an overnight fast was sufficient to provoke robust changes in neural activity, the next stage was to investigate how appetite could modulate pain perception. Using the same paradigm of an overnight fast versus a large breakfast, we also presented photographs of appetising food in an attempt to further enhance hunger-pain interactions. We recorded LEPs obtained under conditions of fasting and satiety, in the context of appetitive food photographs and inedible objects. Subjective pain ratings were initially reduced when participants were hungry compared to when fed, but this effect soon disappeared, probably due to habituation. Source dipole analysis of the LEPs revealed that activity in PHG was weaker during fasting, and activations of the operculo-insular cortex, ACC, PHG and cerebellum were smaller in the context of appetitive food photographs, regardless of appetite status. That pain was temporarily attenuated, and cortical processing of noxious stimuli in pain-related brain structures reduced when participants were fasted or passively viewing food photographs, lends support to a possible interaction between opposing motivational forces of the eating drive and pain.

In order to further explore the effect of hunger on pain we employed the same paradigm of an overnight fast and satiation, and modelled pain-provoked changes in functional connectivity of fMRI data. Using ROIs of areas commonly implicated in pain processing, we found some functional connectivity that was altered by changes in the homeostatic energy balance. During fasting, a saliency network consisting of the anterior insula, ACC, and PFC was more strongly functionally connected. The saliency of any stimuli appears to be enhanced when appetite is heightened, which may be beneficial when seeking food. During satiety, the precuneus and PCC (core areas of the DMN) were more strongly functionally connected, and this enhanced connectivity could be accounted for by differences in subjective hunger ratings between the sessions. We also found that some functional connectivity remained the same regardless of appetite; connectivity probably underlying ascending nociception, descending pain modulation, and integration of pain-induced affect with autobiographical memory, was unaffected by appetite status. These results lend more support to the theory that appetite can modulate neural substrates of pain.

Thus far, while pain processing at the neural level appeared to be robustly modulated by changes in appetite, we could not find reliable long-lasting effects of appetite on subjective pain perception. In Chapter 7, we again employed a paradigm of overnight fasting and satiety. We replaced the photographs of appetising food and inedible objects with a bread odour and a linen odour, closely matched for liking, familiarity, and intensity. We also utilised weak and strong pain stimuli instead of the single-strength pain stimuli described in Chapters 5 and 6. When participants were fasted, weak pain stimuli were rated as less painful whichever odour was presented. Strong pain stimuli, a more salient threat to homeostasis, were also rated as less painful when participants were fasted, but only in the context of the bread odour. It seems likely that an appetising food odour is a more powerful capturer of attention than a pleasant, homeostatically-irrelevant odour, but again, this effect was not long-lasting.

8.2 Potential practical applications

The experimental methods used do not translate easily to chronic pain, due partly to the brief nature of the nociceptive stimuli, and the absence of psychological issues such as depression, which are frequently found to be comorbid with chronic pain (Finan and Smith 2013; Ohayon and Schatzberg 2009). In addition participants habituated quickly to both the laser and the electrical pain stimuli, making ever-decreasing pain ratings after approximately 30 trials of each. It is therefore difficult to predict how appetite or appetitive stimuli would affect long-lasting subjective pain perception, since we could not model it in these studies. We can be more confident that food stimuli may be useful in the context of short, painful events. Ambient appetitive stimuli such as food odours may provide a valuable, inexpensive distraction from short medical procedures like vaccination shots and injection of local anaesthetic. They may be even more beneficial if patients must be fasted before undergoing a procedure, such as for fasting blood glucose or lipid profile testing.

Our neuroimaging results show that homeostatic energy balance manipulations have somewhat longer lasting effects on neural pain processing. While subjective pain ratings indicated participant habituation after approximately 30 trials, EEG and fMRI recordings were usable from the start to the end of the sessions. As described in

Chapters 5 and 6, fasting appears to boost the saliency of any stimuli, food-related or otherwise. This may help to explain how intermittent fasting works as a treatment for chronic pain (a therapy that has been employed for many years in some countries), and why such therapy is reportedly accompanied by increased alertness (Michalsen 2010). Fasting for chronic pain may in fact be augmentable by appetitive stimuli; this remains to be demonstrated.

8.3 Limitations

The most obvious limitation common to Chapters 5, 6, and 7 is the issue with pain habituation and the resultant discarding of usable trials. Our results should therefore be treated with ample caution, since they are based on fewer trial numbers than would be ideal.

Another limitation is the lack of blood serum analysis. Blood glucose sampling was included initially in order to ensure that there was a genuine difference between the fed and fasted states. Comments from a reviewer of Chapter 3 pointed out that other compounds besides glucose are influenced by the homeostatic energy balance. On reflection, it would have been beneficial to test for differences in insulin and ghrelin concentrations between states. Both substances show peak changes in concentration at around the length of time after a meal that our participants were tested. There may be unaccounted-for additional hormonal factors influencing any or all of our results, since each experiment utilised the same paired fasted vs. satiated paradigm.

Finally, there is a problem common to most studies of this nature – the majority of our participants were undergraduate and postgraduate students. There is some evidence that this group is an unrepresentative sample for generalising results to the wider population (Henrich et al. 2010); as stated above, the usage of our results requires caution.

8.4 Suggestions for future research

The most obvious conditions that remain to be investigated are variations in hunger and pain. We began to explore this in Chapter 7, using two levels of pain stimuli, and found that while an overnight fast was sufficient to significantly suppress weak pain, strong pain was only inhibited by the fast plus an appetitive food odour. It would be interesting to investigate the effects of a longer fast. Limited research has been carried out with animals and suggests that pain is still suppressed by a complete or intermittent 24 hour fast (Davidson et al. 1992; de los Santos-Arteaga et al. 2003), but (at least in females) potentiated by a 48 hour fast (Khasar et al. 2003). There is no equivalent research with human participants, and therefore no data available concerning differing pain levels or the effects of appetitive stimuli.

Another evident evolution of the current research would be to investigate the effects of fasting and appetitive stimuli on chronic pain. Our participants were healthy and relatively young; while fasting is used successfully as a treatment for chronic pain in other countries (e.g. Michalsen et al. 2002), there is currently no data available concerning the addition of appetitive stimuli to a fasting treatment protocol. This has the potential to be a useful adjunct to existing therapy.

Finally, there is the question of how connected areas of the pain network described in Chapter 6 exert influence on each other. This is a problem that could be solved with an analysis of effective rather than functional connectivity. Dynamic causal modelling was attempted with this data, but there were far too few trials to model effective connectivity. Further research with at least double the number of trials could help to elucidate pathways of descending nociceptive modulation; if there are such areas over the cortical surface, it may be possible to boost their activity with transcranial magnetic stimulation.

8.5 Concluding remarks

Hunger and pain are powerful homeostatic drives, which compete for a behavioural response when experienced simultaneously. Pain and appetite are represented in a widely distributed network of brain areas including thalamus, insula, cingulate cortex, hippocampus / entorhinal cortex, amygdala, and PFC, and neural pain processing in some of these regions is reliably suppressed by a relatively short overnight fast and appetitive stimuli. In addition, pain-induced connectivity of parts of this network can be enhanced by fasting or satiety, while other connections process pain stimuli similarly regardless of appetite status. Functional connectivities enhanced by fasting include a small network of areas involved in stimulus saliency processing (anterior insula, anterior cingulate cortex, and prefrontal cortex), which may facilitate searching for food. Fasting appears to boost the saliency of any stimulus, food-related or otherwise, while satiety evokes DMN activity even in the presence of pain. While there are still many questions remaining over the effect of fasting and appetitive stimuli on strong or chronic pain, it seems likely that fasting and ambient appetitive stimuli provide effective interference with the neural processing of short-lasting moderate pain.

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